ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

SUGAR REDUCTION IN SOFT COOKIES WITH STEVIA

M.Sc. THESIS

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Department of Food Engineering

Food Engineering Programme

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YUMUŞAK KURABİYELERDE ŞEKER MİKTARININ STEVİA İLE AZALTILMASI

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To my supporting family,

FOREWORD

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November 2015 Ayşe Nur BULUT

(Food Engineer)

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ABBREVIATIONS

ADI	**:** Acceptable Daily Intake
ANS	**:**Annual Nutrion Symposium
EFSA	**:** Europan Food Safety Authority
FAO	**:** Food and Agriculture Organisation
FDA	**:** Food and Drug Administration
FOS	**:** Fructo-ligosaccharide
GRAS	**:** Generally Recognized As Safe
HMF	**:**Hydroxymethylfurfural
JECFA	**:** Joint Expert Committee on Food Additives
OAA	**:** Over All Acceptability
PET	**:** Polyethylene Terephthalate
Reb-A	**:** Rebaudiana A
S. Rebaudiana:	Stevia Rebaudiana
SFI	**:** Solid Fat Index
US	**:** United States
WHO	**:** World Health Organisation
WPC	**:** Whey Protein Concentrate

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SUGAR REDUCTION IN SOFT COOKIES WITH STEVIA

SUMMARY

Sugar content of foods is being reduced due to adverse health effects of excessive sugar consumption. Low sugar and low calorie products can be produced by using artificial sweeteners. However, natural additives are preferred to artificial ones due to possible health effects. Snack foods are one of the main sources of sugar in diet. Among the baked snack foods, cookies are known for their high ratio of sugar in their formula. Depending on the cookie variety, sometimes this ratio can be upto 65% percent of the dry matter. Stevia is a natural sweetener that can be used for sugar reduction. It contains sweet tasting compounds named as steviosides. Rebaudioside A is the sweetest compound present in stevia which is approximately 350-400 times sweeter than sucrose.

In this study, development of reduced-sugar soft cookies by using stevia extract was investigated. Sweet compounds were extracted from dried stevia leaves with hot water as it is the best solvent according to the previous studies in literature. American soft cookie was chosen as a model cookie type. Buttermilk powder was used as a bulking agent and to improve the flavor, color and texture of the cookies. Other ingredients were flour, margarine, powdered granulated sugar, salt, sodium bicarbonate and water. Creamery method of production was used for cookie preparation. Amounts of water, stevia and buttermilk powder and baking duration for each formulation was determined by preliminary experiments. Four formulations were developed in which the sugar content was reduced by 30 and 60% by stevia extract with and without buttermilk powder at a level of 5%. Flour:water ratio was kept constant in all formulations. Baking time was modified to obtain a moisture content of 8% in cookies for all formulations. A control and an only buttermilk powder containing cookies were also produced for comparisons.

Moisture content, water activity, spread ratio, color, textural properties and HMF content of the cookies were measured. Flavor and texture of the cookies were evaluated according to descriptive sensory analysis method. The changes in the properties of cookies after 60 days of storage in polyethylene terephthalate (PET) containers at 25°C were also determined.

Spread ratio of stevia containing cookies was significantly lower than that of control sample. Only buttermilk powder containing sample had the highest spread ratio. Weight loss of cookies after baking differed in accordance with baking duration to obtain 8% moisture content. After 60 days of storage at 25°C, the greatest weight loss was determined in 30% reduced-sugar sample.

Significant decrease in moisture content was observed in all of the cookies after storage. Moisture content of the 60% reduced-sugar sample was similar to that of the control sample. Highest initial water activity was obtained in the 60% reduced-sugar sample. After storage, water activity values of the control and buttermilk powder containing samples were significantly higher than those of stevia containing cookies.

All cookies exhibited lower L* values than the control cookie. b* values of all the samples were close to each other. The lowest a* value was found in control cookie. Addition of buttermilk powder to the cookies resulted in an increase in a* value. Total color change values of the samples were calculated by taking the color values of control sample as reference. Total color change values of both stevia and buttermilk powder containing samples were higher than those of the other samples before storage. Highest total color change value was observed in only buttermilk powder containing sample after storage. Hydroxymethylfurfural (HMF) content of all of the cookies was found lower than those reported for commercial cookies.

Initial hardness values of both stevia and buttermilk powder containing samples were similar to that of control sample. Initial hardness of only stevia containing samples was significantly higher than that of the control sample. Hardness of all of the samples increased significantly after 60 days of storage. Highest work to break down value was determined in 30% reduced-sugar sample.

In sensory evaluations, sweet taste score of control and buttermilk powder containing samples was highest and that of 60% reduced-sugar sample with buttermilk powder was the lowest. Aftertaste score of 60% reduced-sugar sample with buttermilk powder was the lowest. Rest of the samples had similar aftertaste scores. Butter taste score of only buttermilk powder containing sample was significantly higher than those of others. Crispiness of 30% reduced-sugar sample was the greatest among samples. The sample with only buttermilk powder was given the highest overall acceptability score. Both stevia and buttermilk containing samples were found similar to the control sample in terms of overall acceptability. Unlike most of the artifical sweeteners, no metallic or bitter taste was observed in stevia containing samples at levels used in this study.

Use of combination of stevia extract with buttermilk powder in formulation resulted in cookies with similar quality properties to those of the control sample. Therefore, stevia extract and buttermilk powder can be used for production of reduced-sugar soft cookies. Buttermilk powder contributed positively to the flavor, texture and color of soft cookies. However, it should be taken into account that buttermilk powder can undergo browning during cooking. Therefore, its usage level, baking temperature and duration should be adjusted accordingly.

YUMUŞAK KURABİYELERDE ŞEKER MİKTARININ STEVİA İLE AZALTILMASI

ÖZET

Fazla şeker tüketiminin insan sağlığına olan negatif etkilerinden dolayı gıdaların şeker içeriği azaltılmaktadır. Düşük şekerli ve düşük kalorili gıdalar yapay tatlandırıcılar kullanılarak üretilebilir. Fakat yapay tatlandırıcıların olası olumsuz sağlık etkileri sebebiyle doğal tatlandırıcılar tercih edilmektedir.

Günlük diyetteki şeker miktarının büyük bir kısmı atıştırmalık gıdalardan gelmektedir. Fırınlanmış atıştırmalık gıdalar arasında kurabiyeler, formülasyonundaki yüksek orandaki şeker miktarıyla bilinmektedir. Kurabiyenin çeşidine bağlı olarak bu oran kuru madde miktarının %65'ine kadar çıkabilmektedir.

Stevia, şekerin azaltılmasında kullanılabilecek doğal bir tatlandırıcıdır. Steviosid adı verilen tatlı bileşenler ihtiva eder. Steviada bulunan bileşenlerden en tatlısı olan Rebodiosid A şekerden yaklaşık olarak 300-400 kat daha tatlıdır. Bu çalışmada, stevia ekstraktı kullanılarak şekeri azaltılmış yumuşak kurabiyelerin üretimi araştırılmıştır. Literatürde önceden yapılmış çalışmalarda stevianın tatlı bileşenlerinin ekstraksiyonunda en etkin çözgenin sıcak su olduğu tespit edilmiş olmasından dolayı, ektraksiyon işlemi bu şekilde gerçekleştirilmiştir. Ekstraksiyon işleminde kurutulmuş ve öğütülmüş stevia yaprakları kullanılmıştır. Daha sonra çözelti santrifüj ve filtreleme işlemleriyle katı partiküller sıvı fazdan ayrılmıştır.

Model kurabiye çeşidi olarak yumuşak Amerikan kurabiyesi seçilmiştir. Kurabiyelerin tadını, rengini ve yapısını geliştirmek ve hacim arttırıcı olarak kullanmak üzere yayıkaltı suyu tozu seçilmiştir. Yayıkaltı suyu, yağ üretimi sırasında açığa çıkan bir yan üründür. Yayıkaltı suyunun pastörize edilmesi, konsatraste edilmesi ve sprey kurutma işlemi ile kurutulması sonucu yayık altı suyu tozu elde edilir. Lezzet ve dokuya katkısından dolayı fırıncılık ürünlerinde, sağlığa faydalı bileşenlerinden dolayı fonksiyonel ürün yapımında kullanılır. Diğer bileşenler un, margarin, pudra şekeri, tuz, sodyum bikarbonat ve sudur.

Kurabiyenin hazırlanmasında yağ ve şekerin önceden karıştırılarak krema haline getirildiği üretim metodu kullanılmıştır. Su, stevia ve yayıkaltı suyu tozu miktarı ve her bir formulasyon için pişirme süresi ön denemeler sonucunda tespit edilmiştir. Yumuşak Amerikan kurabiyesi formülasyonu şeker miktarını %30 ve 60 oranında azaltacak şekilde iki farklı oranda stevia ekstraktı ve %5 oranında yayıkaltı suyu tozu eklenecek şekilde modifiye edilmiştir. Pişirme süresi, bütün formülasyonlardan elde edilen kurabiyelerde nem oranı %8 olacak şekilde ayarlanmıştır. Bu şekilde, şeker miktarının %30 ve 60 oranında azaltıldığı, yayıkaltı suyu tozu olan ve olmayan şeklinde dört farklı formülasyon geliştirilmiştir. Kontrol formülasyonu ve sadece yayıkaltı suyu tozu içeren örnekler de karşılaştırma yapabilmek amacıyla üretilmiştir.

Kurabiyelerin pişirme ve depolama sırasındaki ağırlık değişimi, nem miktarı, su aktivitesi, yayılma oranı, renk ve yapı özellikleri ve HMF içeriği tespit edilmiştir.

Kurabiyelerin tat ve doku özellikleri tanımlayıcı duyusal analiz ile ölçülmüştür. Kurabiyeler 60 gün boyunca 25°C'de polietilenterafitalat (PET) kutularda depolanmış ve özelliklerinde gerçekleşen değişimler tespit edilmiştir.

Stevia içeren kurabiyeler kontrol örneğinden daha düşük yayılma oranı göstermiştir. Sadece yayıkaltı suyu tozu içeren örnekler ise en yüksek yayılma oranına sahip olmuştur. Pişirme sonrasında gerçekleşen ağırlık kaybı, %8 nem oranı elde etmek amacıyla değişen pişirme sürelerine bağlantılı olarak gerçekleşmiştir. Depolama sonucunda en büyük ağırlık kaybı şeker miktarı %30 azaltılmış örneklerde görülmüştür.

Örneklerin başlangıç nem miktarları beklenildiği gibi benzer çıkmıştır. Depolama sonrasında, bütün kurabiyelerin nem miktarında belirgin bir düşüş gözlemlenmiştir. Şeker miktarı %60 azaltılmış örneğin nem miktarı, kontrol örneğininkine benzer çıkmıştır. Depolama öncesi en yüksek su aktivitesi değeri şeker miktarı %60 azaltılmış örnekte gözlemlenmiştir. Depolama sonrası en yüksek su aktivitesi kontrol ve sadece yayıkaltı suyu tozu içeren örneklerde elde edilmiştir.

Bütün örnekler kontrol kurabiyesininkinden daha düşük L* değerleri göstermiştir. b* değeri ise bütün örneklerde aynı çıkmıştır. En düşük a* değeri kontrol kurabiyesinde ölçülmüştür. Örneklerin toplam renk değişim değerleri kontrol örneğinin renk değerleri referans değerler olarak alınarak hesaplanmıştır. Hem stevia hem de yayıkaltı suyu tozu içeren örneklerin toplam renk değişimi diğer örneklerin toplam renk değişim değerlerinden daha yüksek çıkmıştır. Depolama sonrası sadece yayıkaltı suyu tozu içeren örnek en yüksek renk değişim değerine sahip olmuştur. Bütün örneklerdeki hidroksimetilfurfural (HMF) miktarı, ticari kurabiyeler için rapor edilmiş HMF konsantrasyonlarından daha düşük olmuştur.

Stevia ve yayıkaltı suyu tozunu birlikte ihtiva eden örneklerin başlangıç sertlik değerleri kontrolünki ile benzer bulunmuştur. Sadece stevia bulunduran örneklerin sertlik değerleri kontrolün sertlik değerinden yüksek çıkmıştır. Depolamadan sonra bütün örneklerin sertlik değerinde artış gözlenmiştir. Kurabiye örneğini kırmak için gereken iş değeri en yüksek %30 azaltılmış örnekte tespit edilmiştir.

Duyusal analizde, en yüksek tatlı tat skoru kontrol ve sadece yayıkaltı suyu tozu içeren örneklere verilmiştir. En düşük tatlı tat ve ağızda kalan tatlı tat skoru ise şekeri %60 azaltılmış yayıkaltı suyu tozu içeren örneğe verilmiştir. En yüksek tereyağı tadı skoru sadece yayıkaltı suyu tozu içeren örneğe verilmiştir. En yüksek gevreklik skoru ise şekeri %30 azaltılmış örneğe verilmiştir. En yüksek genel beğeni puanını yayık altı suyu tozu içeren örnek almıştır. Stevia ve yayıkaltı suyu tozu içeren örneklerin genel beğeni puanı kontrol örneğininkine benzer çıkmıştır. Birçok yapay tatlandırıcının aksine, stevialı kurabiyelerde çalışmada kullanılan stevia oranlarında metalik ve acı tatlara rastlanmamıştır.

Formülasyonda stevia ekstaktının yayıkaltı suyu tozu ile beraber kullanımı sonucunda kontrol kurabiye örneğinin özelliklerine benzer özelliklerde kurabiye elde edilmiştir. Bu nedenle stevia ekstraktı ve yayıkaltı suyu tozu şekeri azaltılmış yumuşak kurabiyelerin üretiminde kullanılabilir. Yayıkaltı suyu tozunun yumuşak kurabiyelerin tadı, yapısı ve rengine olumlu katkıda bulunduğu gözlemlenmiştir. Fakat, pişirme sırasında yayıkaltı suyu tozunun esmerleşme reaksiyonuna girebileceği göz önünde bulundurulmalı, kullanım oranı, pişirme sıcaklığı ve süresi buna göre belirlenmelidir.

1. INTRODUCTION

The adverse effects of sugar consumption on human health have been shown by many studies. Both obesity and diabetes increase the risk of cancer and cancer-related mortality, suggesting that excess of energy, insulin, and glucose are all important determinants of cancer growth (Tappy et al., 2010). As a result, the consumers prefer low-sugar or sugar-free products. Substituting sugar with sweeteners such as Aspartame and Neotame, or sugar alcohols like sorbitol and mannitol are good alternatives to reduce sugar amount in products. However, some side effects on liver and brain along with increased leukemia risk of chemically produced sweeteners are on debate (Ashok and Sheeladevi, 2015; Abdel-Salam et al., 2012; Soffritti et al., 2007). For these reasons, natural ways of sweetening products are being searched. Stevia is one of the natural alternatives of sugar. It has sweet taste without any sugar and calorie, which makes it an appropriate ingredient for diabetic and dietetic products. In this thesis, sugar reduction in soft cookies with stevia was studied.

1.1. Purpose of Thesis

In the world, sugar reduction is a trend topic for improving health. The link between sugar consumption and several diseases such as; diabetes, obesity and cancer has been shown in many studies. Authorities are looking for ways to cut down sugar and calorie intake. Snack foods, such as cookies, are great source of sugar. Another trend in the market is towards natural ingredients which includes sweeteners. In this study, sugar reduction in soft cookies with a natural sweetener, stevia, in combination with buttermilk was studied.

1.2. Literature Review

1.2.1. Cookie production

Cookie production basically consists of mixing all the ingredients, forming the dough and baking (Figure 1.1). The method of shaping the dough differs in three ways which are; cutting, rotary molding and extruding or deposing. The difference in the process is due to the dough consistency. Harder cookie dough can be cut. However as the consistency of dough decreases, rotary molding, extruding or deposing are applied.

Figure 1.1. Cookie production.

1.2.1.1. Ingredients

Flour

Flour is the main component nearly in all cookie recipes and made from wheat. There are three main parts to a wheat grain (Figure1.2); the outer seed coat, caned bran; a tiny germ, which would form next year's root and shoot; and the largest component, called endosperm, which is the food storage, intended to feed the young plant during its early growth.

Flour is obtained by grinding entire wheat grain. In this stage, flour is called whole grain flour which is known by its high fiber content due to bran and germ of the grain. Bran and germ contribute color, flavor and nutrition however they do not contribute to the baking process. Separating the germ and brain results in the fine white flour which contains only the endosperm part of the grain and this type of flour is mainly preferred for baking goods. As the water is added to flour to obtain a dough, protein absorbs the water rapidly, and reacts with it. As a result gluten is formed which has an elastic property and can be stretched (Towsend, 1990).

Wheat is categorized as hard or soft based on kernel texture, one of the major determinants of its end use. Because of its hard texture, hard wheat requires more energy to be milled into flour and produces a coarse flour, which yields more starch damage. Vice versa, wheat kernels with softer texture produce finer flour with less starch damage. The majority of wheat grown worldwide is hard (Tanhehco and Ng, 2008). Wheat is also classified according to color of the covering bran as white or red. Even though white wheat is more aimed to sprout under moist and warm conditions, after milling, bran is less bitter than red wheat. Also red bran wheat darkens the flour more than white bran wheat after milling. Red bran wheat is preferred for thickening applications (Lin and Vocke, 2004).

Flour proteins, starch, pentosans and lipids all affect the size or spread of cookies as well as their texture and appearance. So, these components need to be taken into account when selecting flour. For cookie production, soft wheat flour with low protein content is the most favorable. While hard wheats have more dense ptrotein-starch complex with higher amount of protein, milling process causes a greater water absorbing surface area which results with harder texture and smaller diameter (Hazelton et al., 2003; Tanhehco and Ng, 2008).

Since protein content is highly related with hard texture as a result of gluten development, soft wheat flour with low protein content is generally preferred for cookie making. Cookie formulas generally contain high amount of fat and sugar and low amount of water. Mixing weak gluten flour in two or three stages can control minimal gluten development, usually a desired property of cookie dough. The weak gluten and the relatively high quantities of fat and sugar in the dough allow plasticity and cohesiveness without the formation of a strong gluten network (Lai & Lin, 2006). It also affects spread ratio of cookie. Spread ratio is a function of the spread rate and the set time (Abboud et al.,1985).

During baking, minimal gelatinization of starch occurs due to the low water content of cookie dough as shown by differential scanning calorimetry (Abboud and Hoseney 1984). However, damaged starch, with its greater water-holding capability, is known to negatively affect cookie diameter. Donelson and Gaines (1998) increased the damaged starch content of hard and soft wheat flours used to make sugar-snap cookies through the addition of ball-milled and pregelatinized starch. For both of the flours, addition of damaged starch resulted with smaller spread of cookies.

Pentosan is a component present in cell wall with high water absorbing capacity. The soluble pentosan content is higher in white soft wheat than soft red wheat. So extra water should be added to dough when working with high pentosan containing flour (Lai and Lin, 2006)

Fat

Fat source can be vegetable or animal. They can also be combined to obtain a better function or beneficial cost. The functional performance and textural quality of fats, and fat-containing products are determined mainly by the balance between the solid and liquid phases (solid fat index, SFI) and the crystal structures of the solid fats. The SFI affects air incorporation, rheology, mouth feel, shelf-life and other quality issues of many bakery products. The SFI depends on many factors. Some of them can be listed as; degree of hydrogenation and the type of fat. When fat solidify it ends up in three forms of crystalls, which are; alpha (α), beta prime (β'), and beta (β). All the crystals impact hardness, texture, mouth feel and stability. The β form is the most stable, but β' is smoother and has better creaming properties. For these reason β' is preffered over β. The factors that affect the formation of β' crystals include the amount of palmitic acid, the distribution and position of palmitic and stearic acids, the degree of hydrogenation, and the randomization of the fatty acids (Lai & Lin, 2006). Fat dispersion in bakery mixes tends to disrupt the continuity of the gluten chains that form when flour proteins become hydrated. This creates areas of weakness in the structure, resulting in a shorter textured baked goods (Cauvain, 1987). In cookies, fats, shortening and emulsifiers improve flavor and eating characteristics. They provide tenderization, lubrication which prevents the wheat gluten particles from adhering together and retard staling, extention of shelf life by affecting moisture retention, aeration for leavening and volume for providing flakiness in pie crusts, Danish, and puff pastry. They also contribute to development of structure of cakes, icing, and fillings. They modify dough consistency by controlling fat crystal growth and reacting with wheat proteins (Barndt & Antenucci, 1993). Affects on spread ratio. Increasing the shortening level increases spread, especially with partially hydrogenated vegetable oil (Lai and Lin, 2006; Baldwin at al., 1972).

The fats that are mainly used in bakery products are; butter, margarine, shortening and vegetable oil. Butter is produced from milk and gives a premium flavor to desserts. Comparing to margarine, butter spreads and layers more easily and uniformly because of animal fats' physical structure even though both contain the same amount of fat or oil. While vegetable fat used in margarine tends to form in small globules or spheres, while the animal fat found in butter forms crystals (Lai and Lin, 2006). Margarine is manufactured from various hydrogenated animal and vegetable fats (80-85%), with flavoring ingredients, emulsifiers, coloring agents, and other ingredients (Bumbalough, 2000). On the other hand, shortenings (or plastic fats) generally consist of nearly 100% fat; however, there are exceptions, such as roll-in shortening, which may contain moisture (O'Brien, 2000). Vegetable oils have many sources, such as; canola, cocoa butter, coconut, corn, cottonseed, palm, palm kernel, peanut, safflower, soybean and sunflower. For cookie production, shortening (solid or semi-solid) is the suitable type of fat. It contributes to the texture of the porduct by entraping the air and coating the sugar crystalls (Cai and Corke, 2005).

Sugar

Sugar is referred to sucrose, a non-reducing disaccharide which consists of a unite glucose and a unit fructose. Sugar is extracted either from cane or beet. The difference is you can obtain brown sugar from cane with a pleasant taste, but the sugar from the beet is not acceptable unless it is completely white. To obtain brown sugar from beet, molasses of cane sugar (a byproduct of refining) is added to refined beet sugar (Edwards, 2007).

Refined sugar classification is based on the granule size. The most commonly used are regular granulated sugar, also called table sugar. Granulated sugars are further classified into powdered(60µm), ultra-fine(160µm) and extra-fine(200-600µm), depending on the size of the grains (British Sugar, 2010). Fine sugars are preferred because of their better mixing, dissolving, creaming properties. Coarse sugar is suitable for syrups (Lai and Lin, 2006).

As the sugar is one of the main components, quality of cookie is highly related to sugar quantity. It plays a role in controlling batter viscosity, the degree of gelatinization of starch, and the heat setting temperature of proteins. Sugar also serves as a tenderizing agent by retarding gluten development during mixing and affects cookie spread (Indrani and Rao, 2008; Sumnu and Sakıyan Demirkol, 2013). Vetter (1984) found that fine grain size and a high concentration of sugar contribute to significant spreading of the biscuit. Also, smaller crystals will dissolve preferentially in the dough (Manley, 2001). Due to its water holding property, sugar gives a fragile texture to cookie. Also it affects distribution of protein and starch, prevents the formation of a continuous mass (Bean and Setser 1992).

Leavening Agents

The term of leavening describes raising of the dough or batter, which ends up with a greater volume and texture in the end product. Leavening can be achieved by three ways, which are: mechanical, chemical and by the water vapor. In chemical leavening, chemicals such as baking soda, ammonium bicarbonate and baking powder are used. Baking soda liberates carbon dioxide when heated or when mixed with an acid, either hot or cool. The use of baking chemicals such as sodium bicarbonate and ammonium bicarbonate makes biscuits porous and crisp (Indrani and Rao, 2008). In this study, sodium bicarbonate is used.

Water

Water has a very important role in bakery products. The amount and types of dissolved minerals and organic substances present in the water can affect the flavor, color, and physical attributes of the finished baked goods. For example; a dough prepared with soft water may result with sticky dough (Matz 1972). Water has a complex role in biscuits because it determines the conformational state of biopolymers, affects the nature of interactions between the various constituents of the formula, and contributes to dough structuring (Elliasson and Larsson, 1993).

1.2.1.2. Cookie production

In the cookie production soft wheat is used. Cookies are supposed to have low moisture content and high sugar and fat amount. Gluten development is not desired in cookie. At this point mixing process is very critical. Mixing can be performed in two or three steps to inhibit gluten development (Lai and Lin, 2006). Once all the ingredients are mixed and dough is created, it should be shaped in a suitable way. There are three different ways of cookie dough shaping which are; cutting, rotary molding and extruding or deposing. The densest dough is shape with cutting after laminating the dough by several rolling pins. Less viscous dough is suitable for rotary molding in which dough is taken by molds on a rotary cylinder and placed on baking convey. The least viscous dough is shaped by extruding or deposing on the baking convey. Baking temperature depends on the ingredients and desired final product moisture, color and texture. Packaging should be after cooling.

Mixing

The quality of a dough mainly depends on three things. They are; the recipe, the nature of the ingredients used and the degree to which these ingredients are mixed together (Manley and Clark, 2011). The process of mixing mainly works for two main causes. The first one is inter milling of ingredients, and the second one is to obtain certain chemical changes which are necessary for the process (Towsend, 1990).

Mixing requirements depend on the dough type. For example, hard dough such as cracker and bread dough needs kneading for gluten development (Edwards, 2007). However with the short dough such as biscuit dough, sugar and fat amount are very high with a limited amount of water, so the sugar does not dissolve totally. Gluten development is not desired with this type of dough (Towsend, 1990). The problem is solved with two steps mixing, also known as creamery method. In the first step sugar is mixed with fat or the water to dissolve. The energy input can be high at this step. Afterwards the flour is added. At this stage, mixing should not last long to prevent gluten development (Edwards, 2007).

Forming

Forming is the stage where the differences in production occurs. According to the properties of the dough, different forming procedures are applied. The forming of cookie dough basically classified as; cutting, rotary molding and extruding or depositing.

Cutting

In cutting, the critical step is rolling out of the dough, which determines the thickness of the cookie. To inhibit the hardening of the dough and obtain regular cookies, dough should be rolled with 2 or more thinner cylinders (Figure 1.2). After this thinning section, lamination begins. In lamination, dough is folded into two to six layers and passed through two or three cylinders again. By this process, the stress on the dough is distributed more uniformly, and working with gluten makes it more suitable for baking. The shaping of cookies is obtained by molds. Dough gets the desired shape between the mold and the rubber cylinder (Treki, 2008). Scrap dough is lifted away and returned either to the sheeter or, less commonly, to the mixer for reincorporation with fresh dough. The temperature, density and toughness of scrap dough is often different from the fresh dough. For this reason, most of the times, incorporation causes quality control problems. The lesser the amount of scrap dough is better (Manley and Clark, 2011).

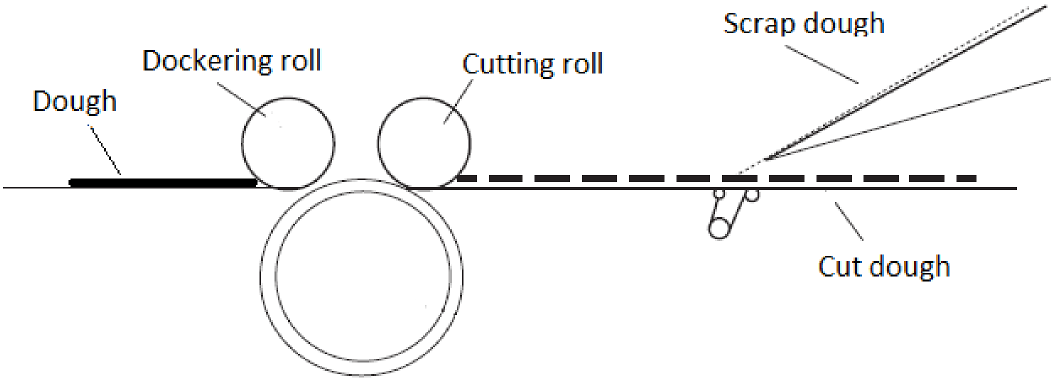


Figure 1.2. Cutting of cookie dough (Manley and Clark, 2011).

Rotary molding

Mostly rotary molder is used for short dough cookies. The dough is forced by a rolling cylinder (A) into the molds, which are in the opposite cylinder (B) (Figure 1.3). The excess dough is scraped by a knife (D) between the two cylinders and the dough in the mold is extracted onto the moving belt. The tip of this blade is below the center line of rolls A and B where maximum dough pressure is exerted in the nip. Roll C is the extraction roll. It has a thick rubber coating. Around Roll C the extraction web (E) passes. The web is cleaned by a scraper (F).

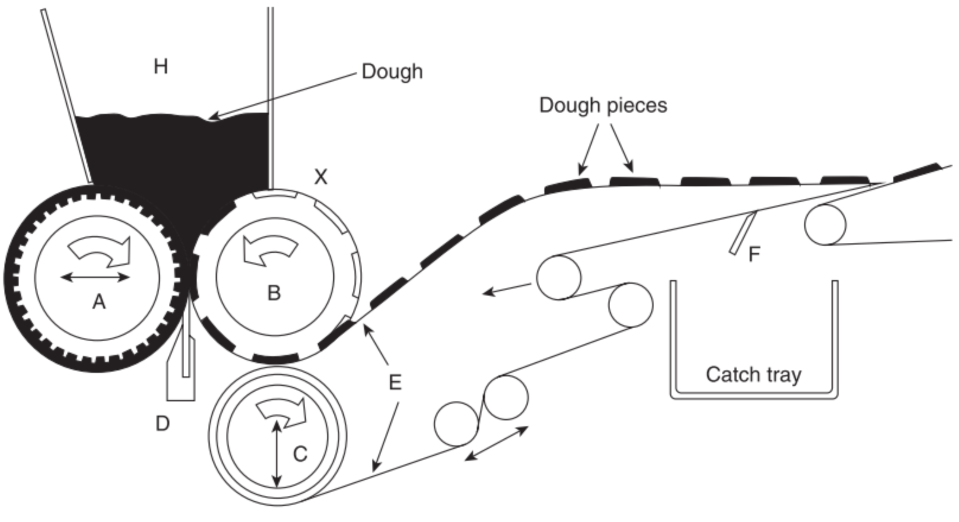


Figure 1.3. Rotary molding of cookie dough. A: forcing roll, B: molding roll , C extraction roll:, D: knife, E: extraction web, F: scraper (Manley and Clark, 2011).

Short dough is also suitable for cutting, however rotary molding has advantages such as;

• there is no need to form and support a dough sheet

• difficulties of gauging are eliminated

• there is no cutter scrap dough which must be recycled. Which is very important as the dough density increases as it is worked and hardens the recycling of excess dough in the case of cutting.

Until recently only short dough could be formed with a rotary molder because of the cohesiveness of the dough. While the knife scrapes the mold, the dough is dragged back resulting with a partially empty mold and faulty shaped dough. However, it is now possible, in a few cases, to rotary mold dough that would not be defined as short. This is possible principally where the area of the mold is small, as in pretzel knots, or the dough is under mixed.

Extruding and depositing

Depositing is a form of extrusion however, the dough for deposition is softer and pourable than extruding, and sometimes it is a batter. The firmest dough will be wire cut with a similar consistency to rotary mold dough. Wire cutting is suitable for sticky dough and also dough that has coarse particles like nuts, chocolate chips, oat flakes and so.

Most machines basically consist of a hopper over a system of two or three rolls which force the dough into a pressure/balancing chamber underneath (Figure 1.4). The rolls may run continuously or discontinuously. Dough is extruded through dies and a frame bearing a strained wire or a blade across the opening of the die holes which cuts the extruded dough at desired length which is determined by the frequency of the blade move. The dough pieces fall onto the band lying under the opening of the extruder. Sometimes the dropped pieces of the dough go under a plastic roller to flatter the surface before the baking. Wire cut dough pieces always have a rough surface which does not change by baking. Also these cookies have a circular form where the center is thicker than the edges. When the cutting part is deactivated, resulting continuous dough pieces form bars or rout cookies. The cutting can be performed before or after the baking according to the dough type.

Depositing is a suitable process for soft almost pourable dough. In depositing instead of die plate, piping nozzles are used. These nozzles may have patterned ends to give strong relief to the extruded dough (Manley and Clark, 2011; Edwards, 2007).

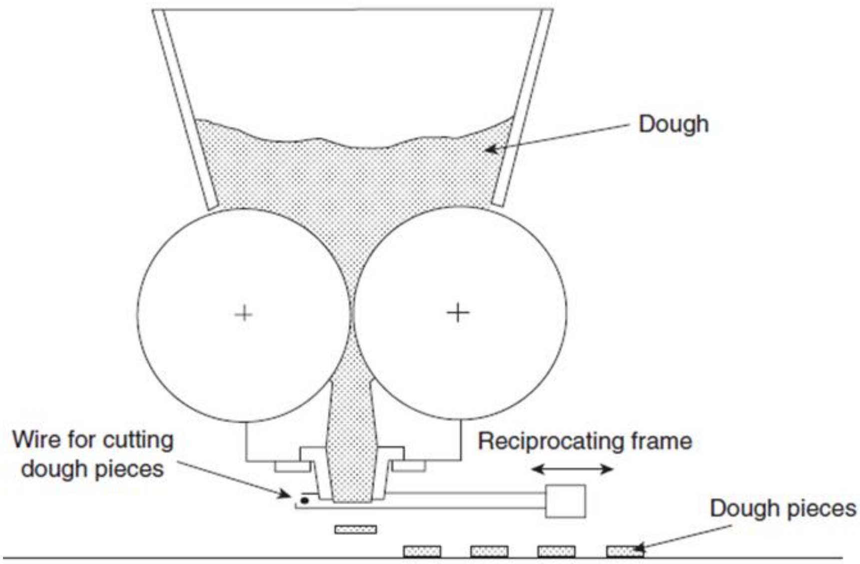


Figure 1.4. Extrusion of cookie dough (Manley and Clark, 2011).

Baking

Cookie baking can be performed with almost all types of ovens, but mostly tunnel ovens, where the cookies are baked on the convey is used. The advantages of tunnel oven are higher throughput and the possibility to arrange the oven in zones. Adjusting the first region cooler than the second region prevents the rapid crust forming which inhibits moisture removal (Edward, 2007).

The physical changes occur to the dough piece during baking are; crust formation, melting of the shortening, steaming of the water, gas expansion, escape of the gases and the steam (Tireki, 2008). The elasticity of the cookie is related to the moisture content of the dough and the humidity of the oven chamber. Shortenings do not have sharp melting points as they are mixtures of compounds. Even though some portions melt, main shortening pockets maintain almost in the original position, so contribute to cookie texture. Even though carbon dioxide evaporates earlier, steam is the main reason of cookie expansion rather than leavening agents such as carbon dioxide and ammonium. All of the expanding gases opens up the mass, increases the volume and strechability of the dough. This action also helps the texture (Tireki, 2008).

Proteins and sugar in the dough, go under maillard reaction, in the presence of heat. Presence of too much alkaline results in alkaline environment, which gives a yellow colored cookie. Proteins start to denaturate irreversibly and gluten starts to gelatinize which gives the cookie its structure (Edward, 2007).

Sugar caramelization takes place and causes brown crust development. Caramelization is the consequence of sugar molecules such as maltose, fructose, and dextrose to produce the colored substances classified as caramels (Treki, 2008).

Cooling

After they come out of the oven, cookies are very soft. It can be stated that starch is still in a gelatinious paste form, dextrins are still in partial solution due to the relatively high moisture content and the shortening at an oil like status rather than fat. Actually all the ingredients are unset yet. When other ingredients lose their water, protein may attract some. Dextrin sets brittle, sugar and shortening crystallize, starch dries out so the cookie has its rigid structure. A rapid cooling may cause cracking due to moisture gradient between center and the edges (Treki, 2008).

Packaging

Packaging is important to maintain cookies as whole and firm. Mechanical strength is the most important criterium for the containers, especially during transportation. Another important criterium is the dimensions of the package. It should be proper to keep cookies as whole. Also packaging material should be vapor proof to maintain cookies firm with desired texture and the shelf life. Tins, waxed papers and polymers are used as packaging materials. (Edwards, 2007).

Packing of cookies can be classified in two as: dump packaging and registered packaging. Dump packaging is mostly suitable for small to medium sized cookies where, the small pieces fall into the pack with no order. Opposite to dumping, in registered packaging, the pieces are kept organized, in an order, both in the package and in the container (Treki, 2008).

1.2.2. Effect of sugar consumption on health

Increased sugar metabolism through glycolysis is associated with cancer and may result from the transformed cell's increased metabolic demand (Onodera et al., 2014). It was claimed that glycosylation, the biochemical process of putting sugars onto proteins and other molecules, is "critically important to many of the signaling pathways involved in turning a normal cell into a cancer cell," by Harvard Medical School biochemist Norbert Perrimon (Alper, 2003).

Both obesity and diabetes increase the risk of cancer and cancer-related mortality, suggesting that excess of energy, insulin, and glucose are all important determinants of cancer growth (Tappy et al., 2010). Epidemiological data support the potential of calorie restriction to enhance cancer prevention (Hursting et al., 2003). Sugar sweetened drink consumption has been associated with increased risk for type 2 diabetes, cardiovascular disease, decreased diet quality (Park et al., 2014) and high lipid level in blood (Zhang et al., 2015). Consumption of low-sugar drinks presented higher fullness feeling than high-sugar drinks (Shearrer et al, 2016).

In 2003, World Health Organization (WHO) recommended to limit sugar intake to 10% of energy intake (WHO, 2003). Brazil signed an aggrement on gradually reducing sugar until 2020, starting from 2013 (de Oliveria Pineli et al., 2016). To improve public health, Belgium published a regulation to tax more on sugar containing foods, drinks and sugar-free and light drinks, starting from January, 2016. In New York City, a regulation was established not to offer or sale sugary drinks in cups and containers that can contain more than 16 fluid ounches (0.5 L)(BBC News, 2012; Kansagra, 2012).

1.2.3. Stevia

Stevia is a semi-woody shrub-like plant which is known by its sweet taste. Some other names of stevia are; honey grass, honey-yerba, Rebaudi's stevia, sweet hemp, sweet herb (of Paraguay), sweet plant (of Paraguay), and sugar leaf, which confirms its sweet taste. Stevia is native to northeastern Paraguay. It is known that Paraguayan Indians have used stevia to sweeten their medicine and drinks, western world met with stevia in the late nineteenth century (Small, 2011). Stevia leaves are approximately 30 times sweeter than sucrose, and some components of stevia approximately 300 times sweet than sucrose (Small, 2011). The sweetness of stevia compounds compared to saccharose can be listed as; rebaudioside A (250–450 times); rebaudioside B (300–350 times); rebaudioside C (50–120 times); rebaudioside D (250–450 times); rebaudioside E (150–300 times); dulcoside A (50–120 times); and steviolbioside (100–125 times) (Crammer and Ikan, 1987). Steviol glycosides are hydrolyzed by gut bacteria to release free steviol; once absorbed, undergo glucuronidation in liver and are then excreted. Each steviol glycoside has unique taste and sweetness profile (Serván et al., 2009). An interesting usage of stevioside is with sodium chloride. It is reported that this combination gives a dish softness, for this reson, it is used in the production of pickled vegetables, dried seafood, soy sauces and miso products (Tadhani and Subhash, 2006; Goyal et al., 2010).Stevia plants can be propagated from cuttings or seed. Since germination rates are poor and seedlings are very slow to establish, it is best grown as an annual or perrenial transplanted crop (Brandle et. al, 1998).

England experimentally cultivated stevia during Second World War in purpose to reduce sugar import (Small, 2011). In late 1970's, a Japanese consortium formed for the purpose of commercializing stevioside and stevia extracts (Kinghorn and Soejarto, 1985). Now stevia holds about one-half of Japan's sweetener market (including sugar). It is used in diet soft drinks, sugarless gum, soy sauce, pickled vegetables, and confectionary products. The countries that improved commercial stevia cultivation are China, Thailand, Taiwan, Korea, Japan, Malaysia, Brazil, Indonesia and to a minor extent in the south eastern United States and southern Canada.

Stevia has some advantages and disadvantages comparing to sugar. Sugar has a role at development of the texture in so many products. In cakes, it gives the elasticity to gluten with egg. It tenderizes the meat, it combines with pectin in fruit to form jelly. However, these goals cannot be achieved only with stevia, in the absence of sugar. For these reasons, some other ingredients, such as; yoghurt, apple sauce, egg white, fruit juice etc. are used with stevia (Small, 2011).

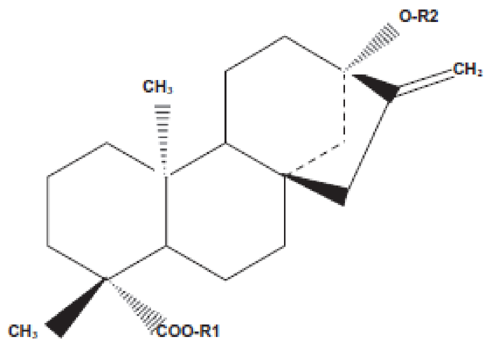
Unlike many low-calorie sweeteners, stevioside is stable at high temperatures and over a range of pH values (Kinghorn and Soejarto, 1985). Stevioside is stable at 95°C which makes it possible to use this sweetener during heating required for food productions. At elevated temperatures and over one hour, the sweetener demonstrated good stability at a temperature up to 120°C, while at temperatures exceeding 140°C it underwent degradation, and its complete degradation occurs when exposed to 200°C (Kroyer, 2010). Steviosides are highly stable in aqueous solutions within a broad range of pH and temperature (Abou-Arab et al., 2010; Panpatil and Polasa, 2008). It was found that aqueous solutions of steviol glycosides are highly stable at pH range of 2-10, which makes it suitable for using as sweetener in carbonated beverages (Kroyer, 2010). In another study it was shown that stevioside possess an excellent heat stability is up to 100°C for 1 h at pH range 3–9, but rapid decomposition occurs at the stevioside possess an excellent heat stability is up to 100°C for 1 h at pH range 3–9, but rapid decomposition occurs at pH level greater than 9 under these conditions

(Buckenhuskers and Omran, 1997). Caramelization occurs when sugar is heated and oxidized. Caramelization does not occur due to lack of sugar, in the case of stevia containing products. It is also non-calorific and non-fermentable (Crammer and Ikan, 1986; Abou-Arab et al., 2010). Stevia does not have water binding property of sugar, which limits the shelf life of products in some cases (Small, 2011).

1.2.3.1. Components of Stevia

The sweet diterpene glycosides of stevia have been the subject of number of reviews (Kinghorn and Soejarto 1985; Crammer and Ikan 1986; Hanson and De Oliveira 1993)**.** However, significant progress towards chemical characterization was not made until 1931, with the isolation of stevioside (Bridel and Lavieille 1931). Eight diterpene glycosides with sweetening properties have been identified (Figure.1.5). The major four are stevioside, rebaudioside A, rebaudioside C and dulcoside A.   The two main glycosides are stevioside, and Rebaudioside A, approximately 2-4% and 5-10% of the dry weight of the leaves. These are the sweetest compounds, however Reb-A is sweeter than stevioside and it has the most desirable flavour profile (DuBois, 2000). The reason is that rebaudioside has more sugar units bound to its steviol aglycone. However, their content in the plant material decreases at the same time (Kovylyaeva et al., 2007). Stevioside is the responsible glycoside of bitter taste, so called "licorice taste". So higher ratios of Reb-A to stevioside is preferred (Crammer and Ikan 1986; Brandle 1999; Oddone 1999).

There are also other related compounds including minor glycosides, such as rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, dulcoside A, dulcoside C and steviolbioside, as well as flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils (Vis and Fletcher, 1956; Erich et al. 1961; Wood and Fletcher 1956; Kodha et al. 1976; Masur et al. 1977; Yohei and Masataka 1978; Crammer and Ikan 1986; Putieva and Saatov 1997; Dzyuba 1998; Dacome et al. 2005; Rajasekaran et al. 2007).



Compound	R1	R2	Sweetness *
Steviol	H	H	
Steviolbioside	H	β-Glc-β-Glc(2→1)	100-125
Stevioside	β-Glc	β-Glc-β-Glc(2→1)	
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) I β-Glc(3→1)	250-450
Rebaudioside B	H	β-Glc-β-Glc(2→1) I β-Glc(3→1)	300-350
Rebaudioside C (Dulcoside B)	β-Glc	β-Glc-α- Rha(2→1) I β-Glc(3→1)	50-120
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) I β-Glc(3→1)	250-450
Rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)	150-300
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) I β-Glc(3→1)	
Dulcoside A	β-Glc	β-Glc-α- Rha(2→1)	50-120

Figure 1.5. Sweet compounds of stevia (Geuns, 2003). *times of sucrose (Crammer and Ikan, 1987).

1.2.3.2. Stevia products

Stevia products can be in various forms. Either direct extraction of the leaf or prufication of the extract to obtain a specific compound of stevia. These extract can be in powder, tablet or liquid forms. Among the stevia forms, powder is the sweetest because it is in the purest form of sweet components. The white powder can be solved in water to obtain a liquid concentrate (Small, 2011). Tabletop type of stevia products consist of Reb A and erythritol mix which is claimed to be 2 times sweeter than sucrose. Tablet forms are mixed with lactose.

Also all-natural soft drinks with stevia sweeteners have been introduced to the market, which is still under development (Bakal, 2011). Stevioside is currently used in sugarless gums in Japan. Although the products lack the sensory qualities of saccharinor aspartame-sweetened products, they enjoy high consumer acceptance in Japan. With the US acceptance of stevia, using it with chewing gums and confectioneries is expected (Bakal, 2011). Redpoint Bio has found that rebaudioside C, enhances the sweetness of caloric sweeteners by up to 50% (Redpoint Bio, 2010).

1.2.3.3. Health aspect of stevia

Stevia extract is known by its diabetic properties (von Schmeling et al. 1977; Alvarez et al. 1981). It has been shown that stevia extract consumption decreases the blood glucose level (Suzuki et al. 1977). Also stevia extract was found to increase glucose tolerance (Curi et al., 1986).

In a study with both obese and healthy humans, sucrose, aspartame and stevia intakes before lunch and dinner meals were compered. It was found that participants did not compensate by eating more at none of the meals they consume aspartame and stevia. Similar levels of satiety were reported compared to when they consumed the higher calorie sucrose preload (Anton et al, 2010).

As a plant, stevia has many human health beneficial compounds. For example, a naturally occurring plant polysaccharide, actually inulin-type fructooligasacchaarides, have been isolated by de Oliveira et al. (2011) from both leaves and also roots of *S. rebaudiana*. This compound is related to prebiotics, dietary fibre, lipid metabolism and diabetes control. Stevia also contains amino acids. Stevia leaves have all the indispensable amino acids with only one missing, which is tryptophan (Lemus-Mondaca, 2012). In a study, five medical plants (*Pueraria mirifica, S. rebaudiana Bertoni, Curcuma longa Linn., Andrographis paniculata (Burm.f.) Nees. and Cassia alata Linn.*) were subjected to antioxidant capacity testing with five different solvents. Among all the medicinal plants, the highest antioxidant capacity was found in *S. rebaudiana Bertoni* (Phansawan and Poungbangpho, 2007).

In a report, it is claimed that Paraguayan Matto Grosso Indian tribes have used stevia as an oral contraceptive. The women were said to drink a daily decoction in water of powdered leaves and stems to achieve this purpose. It is, however, definitely not true that Paraguayan women use it as contraceptive, as an Uruguayan author reported. It is documented that Guarani Indians used "Kaahee"(stevia) before the Spanish troops arrived in Paraguay, according to documents of the National Archive in Ascuncion (Soejarto 1983). An early scientific study claimed that stevia extractions had an antifertility effect in female rats which supports the claim (Planas and Kuc 1968), however, the amount fed to the rats daily was eight times the dosage per kg purportedly used by Paraguayan Indians as an oral contraceptive. Although this report has been frequently cited, its findings have not been reproduced. No effects on fertility were seen in studies on rats or rabbits (Akashi and Yokoyama 1975; Shiotsu 1996). The contraceptive effect of Stevia, if any, does not appear to be due to the sweeteners in it. In one small study, a hot water extract was added to rat food to the level of 0.14% stevioside for 21 days (Akashi, 1975). No reproductive effects were seen in either sex. No endocrine effects were seen in male rats (Sincholle and Marcorelles, 1989), including a study in which 25–30- day-old rats were fed leaf extract for the next 60 days (Oliveira-Filho et al., 1989). This alleged contraceptive activity of the plant has been a controversial issue, but concern has diminished in recent times (Small, 2011).

It has been proved that regular consumption of stevia glycosides reduces insulin, glucose and cholesterol concentrations (Atteh et al., 2008; Anton et al., 2010), enhances the regeneration of cells and blood clotting, inhibits tumor cell growth and strengthens blood vessels (Jeppesen et al., 2003; Barriocanal et al., 2008; Maki et al, 2008). Also they exhibit cholagogic (Kochikyan et al., 2006), anti-inflammatory (Jayaraman et al., 2008; Sehar et al., 2008) diuretic activity, prevent gastrointestinal ulcers (Kochikyan et al., 2006), exhibit antihypertensive (Chan et al., 2000; Lee et al., 2001; Jeppesen et al., 2002), antihiperglycemic (Suanarunsawat and Chaiyabutr, 1997; Jeppesen et al., 2000, 2002; Chen et al., 2006), antirotaviral activity (Suanarunsawat and Chaiyabutr, 1997; Takahashi et al., 2001), exhibit a beneficial effect on glucose (Toskulkao et al., 1995; Suanarunsawat and Chaiyabutr, 1997; Shivanna et al., 2013) and kidney metabolisms (Jutabha et al., 2000).

There are conflicting reports regarding the safety of stevia and steviosides, especially related to male fertility and mutagenicity. But some studies found no correlation between stevia and male fertility (Oliveira-Filho et al., 1989), and also no genotoxic potential was observed for stevia and steviol in a Comet assay (Sekihashi et al., 2002). Infact, stevia tea and stevia capsules are approved for sale in Brazil for the treatment of diabetes (Kinghorn et al., 1985). Physicians in Paraguay reportedly prescribe stevia leaf tea in the treatment of diabetes (Soejarto et al., 1983). With the researches that look for toxic endpoints after giving the substance, no problems have been found with stevioside and Reb A.

1.2.3.4. Regulatory aspect of stevia

Stevia rebaudina extracts containing stevioside are approved as food additives in Japan, South Korea, Brazil, Argentina, and Paraguay, and as of 2009, extracts have also been used commercially in the United States (Small, 2011). The first GRAS (Generally Recognized As Safe) notification in the United States addressed a stevia sweetener rich in rebaudioside A (FDA, 2008), additional GRAS notifications have been published for stevia products that meet Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications and contain at least 95% steviol glycosides (FDA, 2010). These stevia products are finding use in mixed natural sweeteners (Bakal, 2011). Herbal stevia (i.e., raw leaves, usually ground to a powder) is available in much of Europe and elsewhere (Small, 2011).

On March 10, 2010 ANS (Annual Nutrition Symposium) issued a decision on the safety of steviol glycosides prepared on the basis of a proposal from the European Commission (EFSA, 2010). The studies have already showed that substances derived from stevia are not carcinogenic or associated with any other negative effects. Acceptable daily intake (ADI) was established as 4 mg per kilogram of body weight. Then, the European Commission passed a regulation allowing the use of steviol glycosides in 31 different food categories, including beverages, desserts, sweets and sweeteners on November 11, 2011 (Kobus-Moryson and Gramza-Michałowska, 2015). In Turkey, steviol glicosides are allowed on June 30th, 2013 with E960 code, in many products including bakery goods, drinks, yoghurt, ice cream, jam, sauce, chocolates, gums and low calorie confectionaries. Reb A limit in bakery products is 200-1000 mg/kg (Turkish Food Codex, 2013).

1.2.4. Buttermilk

Buttermilk is a by product of butter production which is also called as traditional buttermilk (Yıldız, 2010). 100g of buttermilk contains 0.5 g fat, 3.2 g protein, 3.5 g lactose and 79 g of water (Renner and Renz-Schauen, 1992). In butter production, during churning, milk fat globules are mechanically disrupted and this releases high amount of milk fat globule membrane (MFGM). One-third of MFGM consists of phospholipids (Mulder and Walstra, 1974). Buttermilk is powdered by pasteurization, concentration and spray drying (Augustin at al., 2015). Buttermilk is used in bakery products, sauces, chocolates and chips (Chandan, 1997) and in dairy products such as yoghurt (Tarachoo and Misty, 1998) due to emulsifying property and high water holding capacity of phospholipids in buttermilk (Elling et al., 1996; Lorredig and Dalgleish, 1998; Wong and Kitts, 2003). Consumption of MFGM releases compunds that are called bioactive peptides which have many health benefits such as anti-cancer effects and positive effect on brain (Kidd, 2000; Schmelz et al., 2000; Spitsberg, 2005; Keogh, 2006; Affolter et al., 2010).

1.2.5. Stevia use in sweet products

In a study, sugar used in chocolate was replaced with stevia rebaudioside A and inulin and polydextrose were used as bulking agents. Sugar free chocolate showed similar rheological, melting behavior and texture to reference chocolate but resulted significantly higher Casson viscosity, lower onset temperatures and higher peak widths than the reference sample which offers inulin and polydextrose as suitable bulking agents options combined with stevia (Aidoo et al, 2014).

Other study was performed to obtain sugar free chocolates with stevia (Glucosyl stevioside,a non-bitter Stevia rebaudiana extract) and polydextrose and inulin with different polymerization degrees as bulking agent. Stevia amount was 0.5% for all the samples with 5% maltodextrin. In general, sucrose replacement resulted in a decline of all the color parameters. Chocolate containing inulin with a higher degree of polymerization had higher melting points, greater plastic viscosity and an increased flow behavior index. This might be a result of inulin with higher degree of polymerisation and their crystallization (Shah et al, 2010).

Stevia extracts were also studied in fermented products. In a study, commercial plain low-fat yogurt flavored with natural vanilla was stdied. Three different levels of sucrose, aspartame, erythritol and 95% rebaudiana A stevia sweetener mixture, maltodextrin and 95% steviol glycosides mix and steviol glycosides and a cold water stevia extract were used in the study. The findings underline the importance of careful selection of stevia type and concentration as well as optimizing yogurt cultures and fermentation conditions. The tests revealed that stevia sweeteners mainly influenced the perception of 2 key sensory attributes; namely, sweetness and sourness. (Narayanan et al, 2014) Results showed that maltodextrin and steviol glycosides mix is the most effective (sufficient at 0.7% level).

Another fermented product, subjected to sugar reduction study was strawberry-flavored Petit Suisse cheese. Through microbiological analysis, the ideal sucrose concentration and equivalent concentrations of different sweeteners (aspartame, Neotame, sucralose, stevia, and refined sugar) and their impacts on starter and probiotic cultures were determined (Esmerino et al, 2013). The ideal sucrose concentration was determined using the just-about-right (JAR) scale, and the equivalent concentrations of the sweeteners were subsequently determined by the magnitude estimation method. Microbiological analyses were also carried out to check the viability of the cultures during the product's shelf life. Among the sweeteners, Noetame showed the greatest and stevia showed the least sweetening power however it did not exerted a negative effect on the viability of the starter or probiotic cultures.

A similar result is obtained by Morais and his friends (2014), where different sweeteners are compared in chocolate dairy dessert. Sensory analysis results showed that Neotame has the highest and stevia with 95% rebaudioside has the least sweetining power.

To investigate the effects of temperature and fat content on the sweetening effect of sugar and other sweeteners, use of neotame, aspartame, neosucralose, sucralose, and stevia (95% rebaudioside A), in a chocolate milk beverage and sucrose as reference were investigated (Cruz and Bolini, 2014). The study results showed that as the consumption temperature of whole milk increases ideal sucrose concentration decreased. But this effect was not observed with skim milk beverages. Decrease in sweetening power was observed for all of the sweeteners analyzed under the same conditions. Effect was particularly pronounced with neotame and stevia. The decrease was less evident with aspartame and neosucralose. These results showed how important the food matrix and consumption temperature were in terms of sweetness perception.

Performance of different sweeteners were compaired in eight different milk chocolate formulations (Palazzo and Bolini, 2014). Four milk chocolates (sweetened with sucrose, sucralose, rebaudioside and neotame) and four soy-based chocolates, using soy extract as milk replacement were prepared. The multiple time-intensity analysis was performed to determine sweetness, bitterness, chocolate flavor and melting rate by evaluating them in separate instances with an intensity reference. There was no difference found between the sweeteners and sucrose. With respect to chocolate flavor, rebaudioside in the milk chocolate did not differ significantly when compared to sucrose for any of the parameters analyzed.

The highly intense sweet flavor of stevia was showed by a study (Edelstein et al., 2007) where the sugar substitution was performed by xylitol, maltitol, isomalt, mannitol, sucralose and stevia at the levels of 25, 33, 50, 100% in cupcakes. Then cup cakes were compared for rise, appearance, texture, tenderness, and flavor in sensory analysis. According to results, stevia was not a suitable replacement for sugar in baking as a one to one gram replacement of table sugar. Desirable quality characteristics of cake are not present with any percent stevia replacement. Only with the 25% replacement brownish color on the surface is obtained.

In another study sugar was replaced with sorbitol and stevioside mixture in fenugreek cake made with 10% debittered fenugreek seed powder, polysorbate 60 as the emulsifier and xanthan as hydrocolloid. It was reported that, the combination ended up with an acceptable cake (Manisha et al, 2012).

Sugar reduction with stevia extracts was studied in muffins. In the study of Zahn et al. (2013), sucrose was replaced at level of 30% with iso-sweet amount of rebaudioside A with several fibers including inulin and polydextrose. The combination of inulin or polydextrose with rebaudioside A resulted in products with characteristics close to those of the reference. The incorporation of these replacers reduced energy by 6 or 5 kJ/100 kJ, and increases fiber content from 1.3 g/100 g to 4.6 or 7.1 g/100 g, respectively.

In a study to obtain low calorie and high protein cookies, 15, 20, 25 and 30% of sugar was replaced with stevia leaf powder on %sweetness basis and wheat maida flour was substituted with defatted soy flour at the levels of 10, 15, 20 and 25% (Kulthe, 2014). As the level of stevia leaf powder was increased, hardness of the cookies significantly decreased. Also decreases in diameter, spread ratio and spread factor were observed due to the decreased level of sugar crystalsin sweetener containing formulations which was expected to melt and cause spreading action. This result also showed by Abboud et al. (1985). The sensory evaluations showed that 20% substitution of both defatted soy flour and stevia leaf powder did not affect overall acceptability of the cookies.

In another study, sugar reduction of short dough cookies was performed by substituting with fructo-ligosaccharide (FOS) at the levels of 40, 60 and 80% of sugar. Sensory evaluations indicated that the panelist liked the FOS-containing cookies (Handa et al., 2012). FOS is a prebiotic soluble fiber and a low calorie sweetener. Diameter, height, spread ratio, hardness, moisture of the cookies, sensory attributes and acidity of the extracted fat are analyzed. FOS containing cookies were found to be larger in diameter than control cookies made with sucrose. Panelists liked FOS cookies (up to 60% replacement) over control due to improved color, texture and appearance.

Shelf life of the biscuits with 50 and 75% sugar reduction with stevioside was studied. The authors suggested that the biscuits can be stored in the carton with a polymeric covering if the relative humidity is not over 75% . For relative humidity values above 75%, vapor-proof containers were found necessary. Biscuits with 75% sugar reduction showed the least stability due to high water activity compared to others (Diukareva et al., 2014).

The effect of whey protein concentrate (WPC) at levels of 0, 20, 25, 30, 35 and 40% on biscuits is investigated (Parate, 2011). Results showed that as the level of WPC increased, diameter of the biscuits decreased and thickness increased as a result of shrinkage. Appearance score decreased as the WPC was level increased, especially for 35% and above addition. Darkness of biscuits also increased with WPC level. It was shown that maximum 25% WPC incorporation can make protein rich biscuits without affecting overall quality.

1.3. Hypothesis

Cookies contain high amount of sugar which is known for its adverse health effects. Amount of sugar in cookies should be reduced for lowering its adverse health effects. Stevia is a natural sweetener that can be used for sugar reduction in cookies. However a filler is needed in this case for making up the mass, having light-brown surface color and water holding which are the functions of sugar in cookies. Buttermilk powder can be used as a filler. It is hypothesized that stevia in combination with buttermilk can be used for reduction of sugar in soft cookies.

2. MATERIALS and METHOD

2.1 Materials

All purpose wheat flour (Eksun Gıda, Tekirdağ, Turkey), margarine, granulated sugar (Keskinkılıç Gıda, Kocaeli, Turkey), salt, sodium bicarbonate, dried stevia leaves (Naturel Gıda San. Ltd. Şti, İstanbul) were purchased from local shops. Buttermilk powder (8% fat, 30% protein, 55% lactose and 7.5% ash) was procured from Enka (Konya, Turkey). Sugar and dried stevia leaves were ground to pass through 0.45 mm mesh size.

2.2. Methods

2.2.1. Stevia extraction

Dry stevia leaves were ground in a blender and screened through a screen with a mash size of 0.45 mm to enhance the extraction. Grounded leaf (2.36 g) was extracted with 100 mL distilled water at 70°C for 50 minutes. A water bath was set up on a magnetic stirrer to justify temperature, and the extraction was performed in the water bath. (Das et al., 2014). Than the mixture was centrifuged (Sigma, 2-16PK, Germany) at 25°C at 1940xg for 15 min. The solution was filtered through Whatman No.1 filter paper to separate the extract from the particles. Extract for each formulation was prepared prior to the day of baking and kept at 4°C until use.

2.2.2. Cookie preparation

Preliminary experiments were performed to determine the amount of stevia extract, sugar, buttermilk powder and water to be used. Sugar content of soft cookies was reduced by 30 and 60% by replacement with 7.5 and 15 mL stevia extract, respectively. Buttermilk powder was used at level 5% of dry matter not to brown cookies excessively and to have an observable effect. As a result of preliminary experiments, five formulations with stevia and buttermilk powder were developed (Table 1). A control sample without buttermilk and stevia and a buttermilk-containing sample without stevia were also prepared for comparison. Samples were prepared in triplicates.

Creamery method was used for the dough preparation. Margarine was mixed with powdered sugar (0.45mm) for 3 minutes by a hand mixer (PK-6000, Raks, Istanbul, Turkey), the bowl was scraped every min. In another container, all purpose flour, carbonate, buttermilk powder and salt were mixed with a hand mixer for 1 min. The mixture was added to the sugar-margarine cream through sieving to obtain a homogeneous dough. The mixture was mixed for 1 min with the mixer. Then water and the stevia extract were added and mixed for another 50 seconds with the mixer again. The flour amount was increased to compensate for the solid loss which resulted from the sugar reduction. Water amount was determined according to the flour amount, so that flour/water ratio of the control formulation was obtained in all samples. The dough was scooped with No 40 scoop and flattened with a rolling pin by back and forth movement according to the Standard Approved Method 10-50 (AACC, 2000). Five cookies from one formulation were baked each time on a baking sheet at 200°C in the middle row of a conventional oven (Luxell, LX-3580, Turkey). Standardization of baking time for each formulation was determined through preliminary experiments to obtain 8±0.6% moisture content in baked cookies. Cookies were cooled at room temperature for 25 min and then stored in closed PET (polyethylene terephthalate) packages at room temperature (25°C) in an oven, under dark.

Table 2.1. Sample formulations.

Cookie type	Control	1B*	1S**	1B+1S	2S***	2S+1B
Wheat flour (g)	100	90	115	105	130	120
Buttermilk powder(g)	-	10	-	10	-	10
Sugar(g)	50	50	35	35	20	20
Stevia extract(g)	-	-	7,5	7,5	15	15
Margarine(g)	40	40	40	40	40	40
Salt (g)	1	1	1	1	1	1
Carbonate(g)	1,5	1,5	1,5	1,5	1,5	1,5
Water (g)	30	30	34,5	31,5	39	36
Baking duration (min)	20	20	35	33	40	40

* 5% buttermilk powder, ** 30% sugar reduction, *** 60% sugar reduction

2.2.3. Weight loss

Cookies were weighed before and after baking to determine weight loss during baking. They are also weighed before and after storage to determine weight loss during storage. Weight loss was calculated as difference in weight as percent of initial weight.

2.2.4. Spread ratio

Spread ratio was measured according to the standard AACC 10-50 method (AACC, 2000). Five cookies were aligned side by side and total width was measured. Total width was divided by the number of cookie. The cookies were rotated a quarter and width was measured again. This protocol was repeated for three times. Thickness of cookies was measured as the total height of five cookies. The order of cookies was changed randomly and total height was measured again. The average of the two measurements was calculated and divided by the number of cookies to obtain thickness. Width was divided by thickness to calculate the spread ratio.

2.2.5. Color measurement

Color measurements were done by a chromameter (CR-400, Konica Minolta, Tokyo, Japan) at three equally distant points of cookie from center and edges). Before measurements, calibration with white plate was performed. L* indicates lightness/darkness, a* indicates redness/greenness and b* value indicates yellowness/blueness. Total color change of samples according to control in the day of baking and during storage were calculated with equation 2.1.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^{\,2}} \quad (2.1)$$

2.2.6. Texture analysis

Texture analysis was performed with a texture analyzer (TAPlus Texture Analyzer, Lloyd Instruments, Chichester, England) and analysis software (NEXYGENPlus Materials Testing Software, Lloyd Instruments, Chichester, England). Penetration test was performed by using the single hardness method of the instrument. Measurement was made at the center of the cookies sample. A depression limit of 10 mm, 0.5 mm/sec crosshead speed and 15 gf trigger force were used. Maximum force measured was taken as the hardness of the sample. Break work was also calculated by the software. Texture measurement was performed in triplicate samples from each formulation.

2.2.7. Moisture content

Moisture content of cookies was measured by an infrared moisture analyzer (MOC63u, Shimadzu, Kyoto, Japan) (Hadnadev et al., 2015). Samples were ground in a blender and 1 g of ground cookie was used. Moisture content of two cookies from each formulation was measured and measurement repeated for two times for each cookie.

2.2.8. Water activity

Water activity was measured with 5.5 g of ground cookie with a water activity meter (MA 410171, Protimeter, Marlow, England) at room temperature. Measurements were repeated for two times for each formulation.

2.2.9. Sensory evaluation

1B, 1S, 1SB, 2SB samples and control cookies were evaluated by a ten member educated panel consisting of academic staff of the Department of Food Engineering, Istanbul Technical University, Istanbul, Turkey according to descriptive sensory analysis method (Handa et al., 2012). Analysis was repeated twice. Taste and textural attributes of samples were scored on a seven-point scale (Appendix A). Taste attributes were buttermilk flavor, sweetness, bitterness, saltiness, metallic taste, sweet after taste and overall acceptability. Textural attributes were surface color, surface roughness, inner color, inner particle size, hardness, crispiness, crumbliness in the mouth and cohesiveness in the mouth. The panel was conducted at room temperature in a sensory room equipped with white fluorescent light. Sensory evaluation score forms were prepared and all of the cookies except 2S, was evaluated by panelists. 2S samples were found unacceptable as they were tasteless and hard in a preliminary evaluation. Sensory evaluation procedures were explained to the panelists before testing in each panel. Panelists were asked to read through the instructions on the sensory form. The meaning of each attribute was explained to the panelists to avoid misinterpretation. The panelists were given time to ask for clarification of the sensory evaluation procedure when uncertain or unclear about the process. Water at room temperature and dietary unsalted crackers were provided to clean the mouth after each evaluation.

2.2.10. HMF analysis

The cookies were ground in a blender and 1 g was taken for analysis. HMF was analyzed by high-performance liquid chromatography (HPLC) by using the method of Ameur et al. (2007).

The ground sample (1 g) was homogenised in water and stirred for 5 min with a magnetic stirrer. HMF was extracted by addition of 2.5 ml 40% (w/v) trichloroacetic acid. The mixture was thoroughly stirred for 5 min and the volume of suspension was adjusted to 25 ml with water, stirred for another 5 min and centrifuged at 4000 rpm for 10 min. An aliquot of the supernatant (1.5 mL) was filtered through a 0.45 μm nylon filter (Waters, Massachusetts, USA) and 20 μL of filtrate was analysed by RP-HPLC. An HPLC system (Waters 2695, Waters, Massachusetts, USA) and PDA detector were used. HMF was separated on a C18 Hypersil column (Supelcosil LC-18, Supelco, Pennsylvania, USA) by using methanol: sodium acetate (0.04 M) (20:80) as mobile phase. HMF was detected at 280 nm. All analyses were performed in duplicate. Results are expressed as mg/kg cookie.

2.2.11. Statistical Analysis

Analysis of variance (ANOVA) of factor were performed (SPSS 16, IBM, New York, USA). Means were compared by Duncan Test. Significant value of 0.05 was used. p-values of the factor are shown in Appendix E.

3. RESULTS and DISCUSSION

Effects of stevia, buttermilk powder and storage time on quality characteristics, HMF content and sensory properties of cookie samples were statistically analyzed. Detailed results of ANOVA and statistical comparisions of means are shown in Appendix E.

3.1. Spread Ratio

Spread ratio is an important quality criterion for wire cut cookies. Spread ratio is highly dependent on sugar content. The greatest spread ratio was obtained in 1B samples, which is even higher than that of control cookies (Figure 3.1). An interaction between stevia and buttermilk powder on spread ratio was obtained. 2SB cookies had the lowest spread ratio. A low spread ratio results in a thick cookie. Same results were also reported for sugar-snap cookies (Pareyt et al., 2009), chocolate chip cookies (Bramlett., 2012) and stevia containing cookies (Kulthe et al., 2014). Spread ratio of fructoligosaccharide containing cookies were found to be greater than that of control cookies due to high solubility of this sweetener in water (Handa et al., 2012).

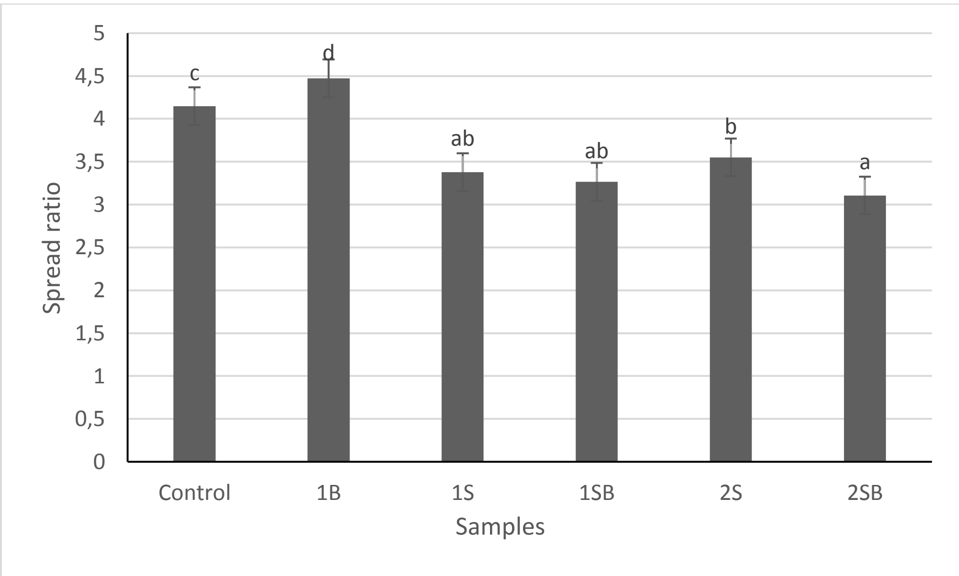


Figure 3.1. Spread ratio (width/thickness) of cookie samples. a-e: Means shown with different letters are significantly different (P<0.05)

3.2. Weight Loss

Weight loss of cookies during baking was measured by weighing the cookies before and after baking. Amount of water in the formulations of cookies was adjusted to obtain the water/flour ratio of control cookies (Figure 3.2). Baking duration was also adjusted to obtain s a moisture content of 8±0.5 %. Weight loss values of samples were as expected from the amount of water in the formula and baking duration.

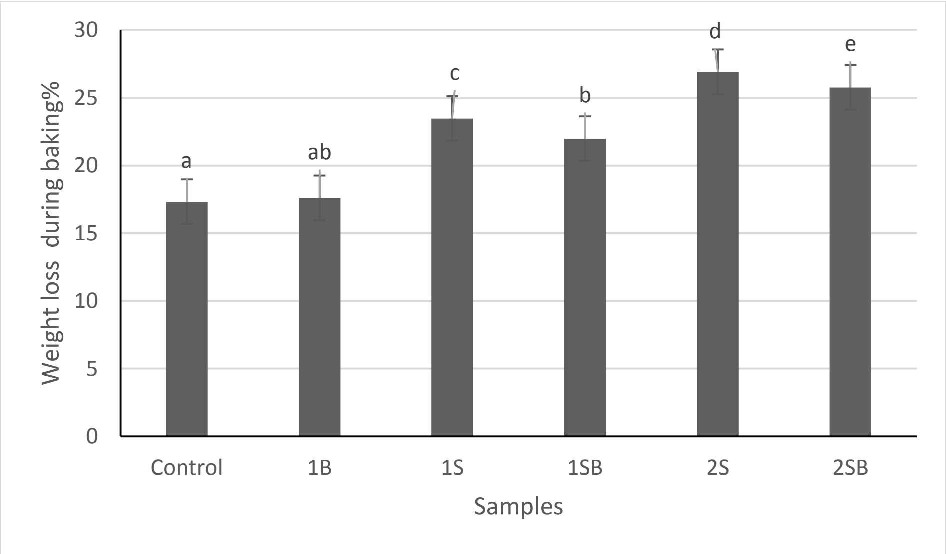


Figure 3.2. Weight loss% of samples during baking. Means shown with different letters are significantly different ($P<0.05$)

Weight of cookies was also measured before and after storage to determine weight loss during storage. Weight loss during storage resulted from moisture loss. The results showed that the greatest weight loss occurred in 1S samples (Figure 3.3). When the buttermilk powder was added to the formula, a significant decrease in weight loss was observed. This could be the outcome of water binding properties of buttermilk powder. Also when compared with the control samples, 1B showed lesser weight loss. The least weight loss was obtained with 1B and the greatest weight loss was obtained with 1S samples.

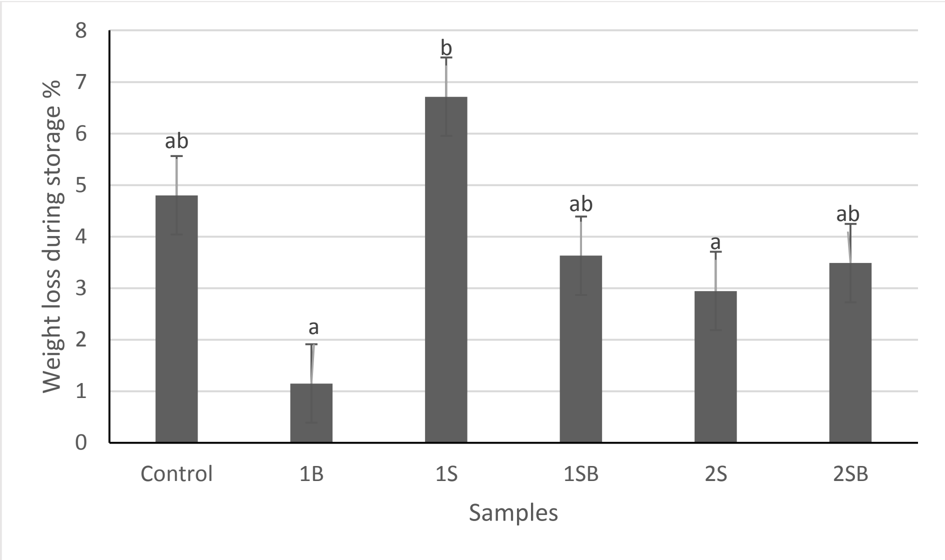


Figure 3.3. Weight loss of cookies after storage at 25°C for 60 days. Means shown with different letters are significantly different (P<0.05)

3.3. Moisture Content

Moisture content of the cookies on the day of baking was similar to each other, which was the target in the baking process. After storage, all of the samples lost significant amount of moisture (Figure 3.4).

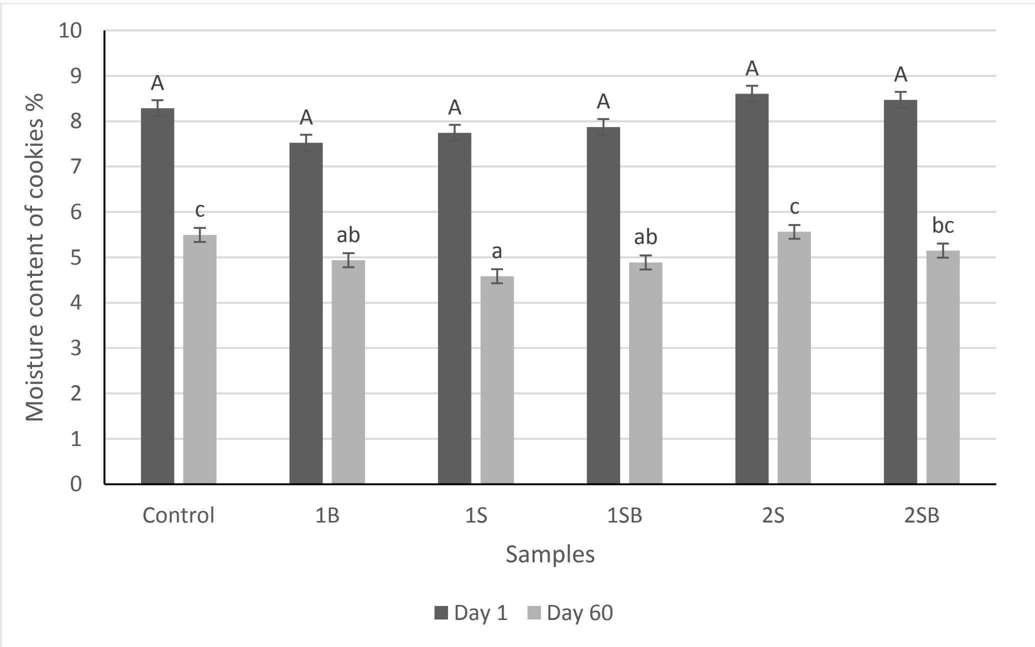


Figure 3.4. Moisture content of cookies. A-E: Means shown with different letters in the day 1 are significantly different (P<0.05) a-e: Means shown with different letters in day 60 are significantly different (P<0.05)

3.4. Water activity

On the day of baking, greatest water activity was obtained in 2S samples (Figure 3.5). As the flour/water ratio of formulations was kept constant, stevia extract became an extra source of free water and increased the water activity. Stevia factor, significantly affected the water activity of samples. Water activity increase by sugar replacement was also obtained in another study in which substitution of sugar was performed with sucralose/maltodextrin:isomalt blend in chocolate chip cookies (Bramlett et al., 2012). After the storage, all the samples with stevia had similar water activity (Figure 3.5). Water activity values of control and 1B samples were significantly lower than stevia containing cookies due to water binding property of sucrose and lactose. The samples with buttermilk powder showed lesser decrease in the water activity. In contrast to the results of this study, de Almeida Marques et al., ovserved increased water activity with increased whey powder content in sugar cookies (2016). This could be a result of difference in whey powder and buttermilk content. Buttermilk contains high amount of phospholipids which binds water and dereases the water activity.

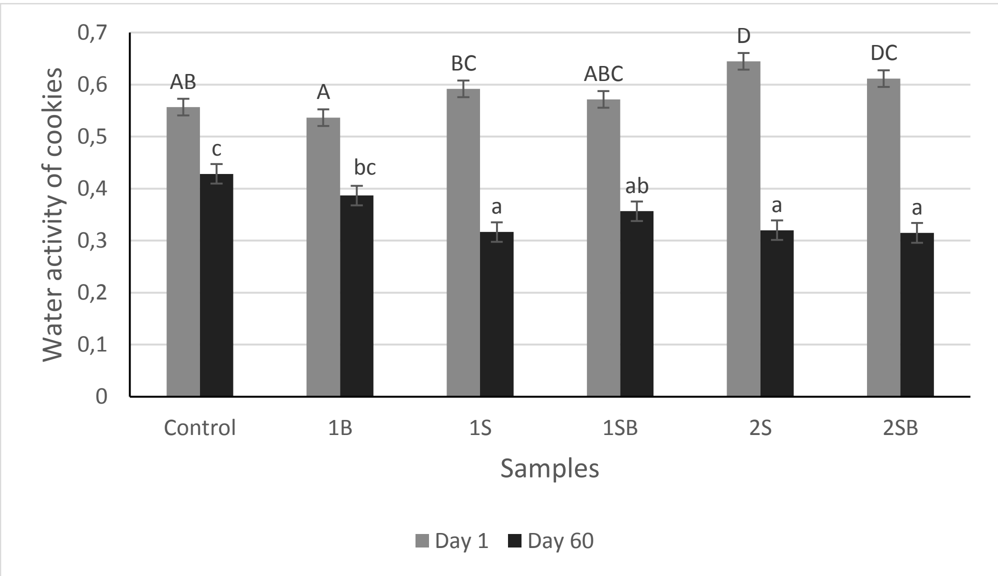


Figure 3.5. Water activity of cookies. A-C: Means shown with different letters in the day 1 are significantly different (P<0.05) a-c: Means shown with different letters in day 60 are significantly different (P<0.05)

3.5. Color Measurements

All samples exhibited lower L* values than the control biscuit (Figure 3.6). b* values of all the samples were close to each other. The lowest a* value was found in control cookies. Also when buttermilk powder was introduced to the formula, an increase in a* value was observed in all samples. L* values of buttermilk samples (1SB and 2SB) were significantly lower than only-stevia containing samples (1S and 2S). Also, L* values of 1S and 2S samples were not different. Stevia amount did not affect the L* value. According to the total color change calculation and statistical analysis, 1SB and 2SB were significantly different than control samples. There are two reasons for color development in cookies. One is caramelisation of sugar in the presence of heat and the other is maillard reaction which takes place in the presence of carbohydrate, protein and heat. Maillard browning reaction between proteins and reducing sugars, contributed significantly to browner/darker product color. Similarly whey powder containing cookies were found darker than control (Secchi et al., 2011). Increase in a* value indicates an increase in the redness which can be the result of browning.

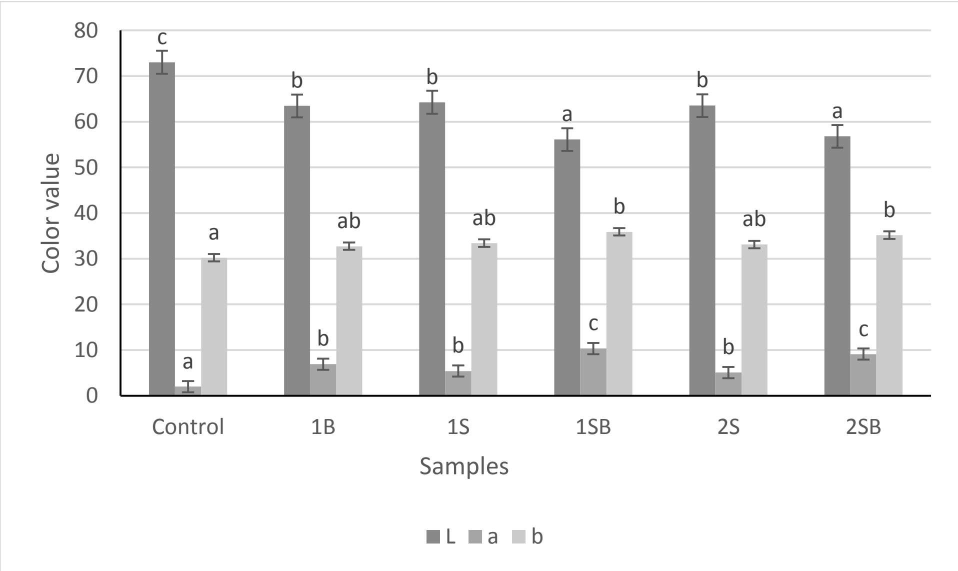


Figure 3.6. Color measurement of samples on the day of baking. All the color values (L, a, b) were evaluated in each other.

After storage, L* and b* values of all of the samples except 1B sample, was significantly higher than baking day (Figure 3.7). However, there was not a significant total color change (Appendix B).

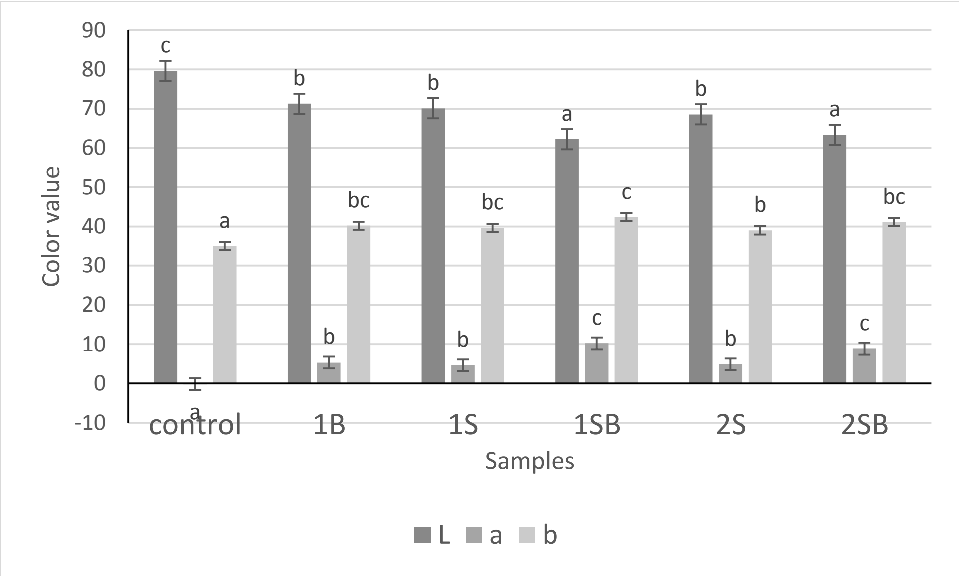


Figure3.7. Color measurement of samples after storage at 25°C for 60 days. All the color values (L, a, b) were evaluated in each other.

3.6. Texture Measurements

The hardness values of the samples on the day of baking were close to each other (Figure 3.8). However, there was an increase and decrease obtained when stevia extract and buttermilk powder was introduced to the formula, respectively. An interaction between buttermilk and stevia on hardness and work was obtained by statistical anaylsis. The closest hardness value to that of control cookie was obtained in 1BS samples. Hardness of 2SB sample, both before and after storage, was similar to control. Hardness of 1S and 2S samples was significantly different than control sample. There was not a significant difference between hardness of 1S and 2S samples. However in the study of Kulthe et al. (2014), as the stevia level was increased, hardness of cookies decreased. There was an increase in hardness of all of the samples after 60 days of storage. This could be the result of moisture loss of the cookies. Before the storage the lowest value of hardness was obtained with 1BS samples, however after the storage the least hard cookie was 2SB, which can be the result of sugar reduction. Incorporation of buttermilk powder also resulted with decreased hardness value, due to its water holding property. Xylitol to cookie shelf life by means of texture was observed by Winkelhausen et al. (2007) and Torres et al. (2013).

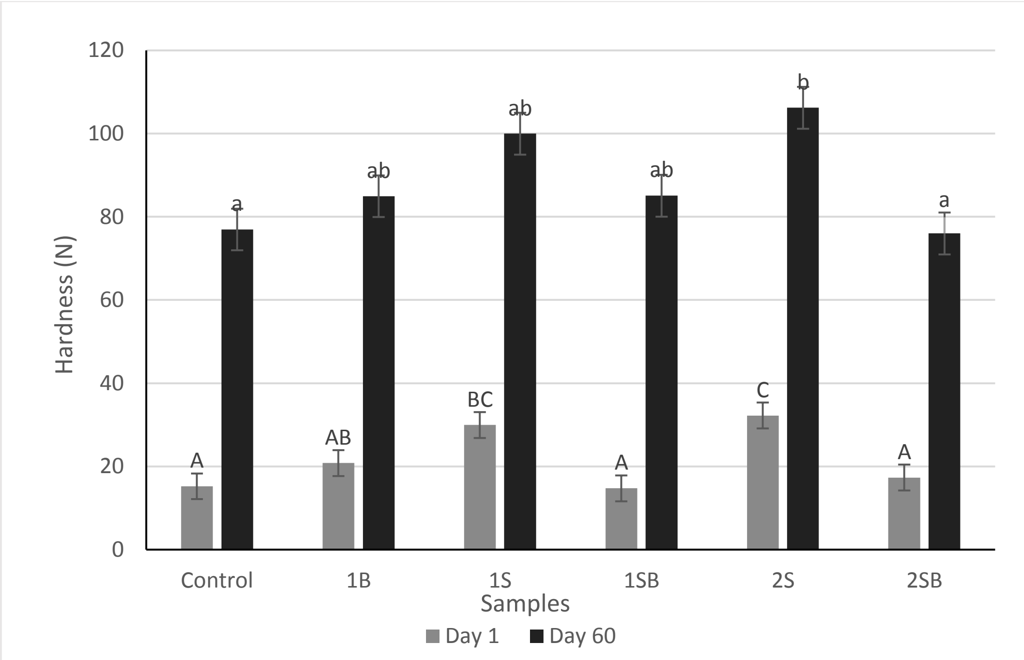


Figure 3.8. Hardness of cookie samples. A-C: Means shown with different letters in the day 1 are significantly different (P<0.05) a-b: Means shown with different letters in day 60 are significantly different (P<0.05)

In the day of baking, work to breakdown 1S and 2S samples was significantly higher than the control (Figuge 3.9). Introducing buttermilk powder to the formula, decreased the work. 2S sample had the highest work value. After the storage, all of the samples' work for breakdown value increased, and was similar.

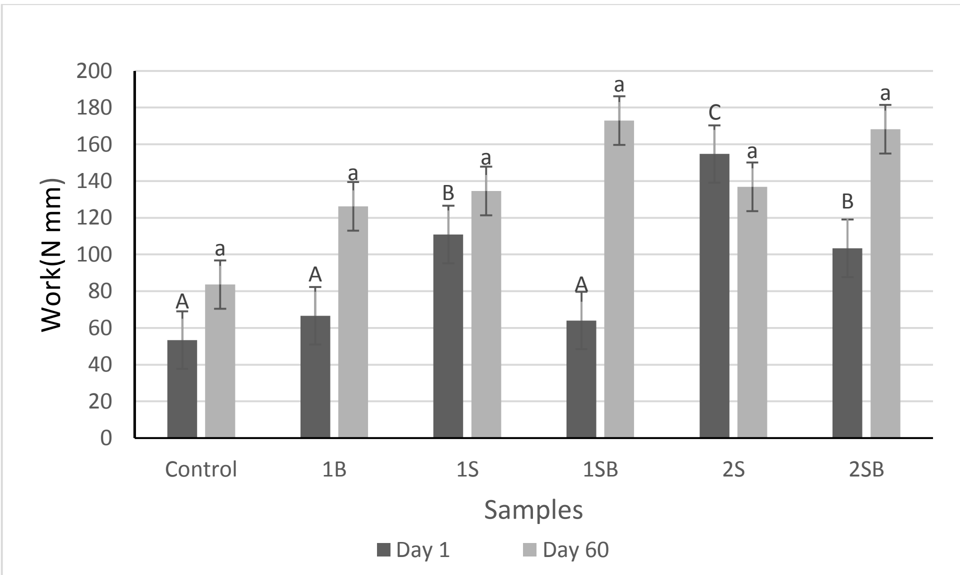


Figure 3.9. Work for breakdown of cookie samples. A-C: Means shown with different letters in the day 1 are significantly different (P<0.05) . a-c: Means shown with different letters in day 60 are significantly different (P<0.05)

Cookies were stored in PET containers as commercial cookies at 25°C for 60 days. Moisture loss resulted in hardening of the texture and loss of crispness of cookies. This hardening is the main limiting deterioration for cookies.

The storage study showed that the PET containers, by itself, is not enough to prevent moisture loss of cookies at 25°C for 60 days. More effective sealing process, an inner package or a different material with more effective vapor-proof property can be more suitable for cookie packaging. In the study of Diukareva et al. (2014), stevioside-containing biscuits were suggested to be kept in carton with a polymeric covering, under a maximum relative humidity of 75% to prevent hardening and preserve texture quality.

3.7. Sensory Evaluation

Butter taste of 1B sample was significantly higher than those of other samples (Figure 3.10). 1SB and 2SB had the lowest butter taste score. However, increasing stevia amount did not effect the butter taste. Sweet taste of 1B was the greatest and 2SB was the lowest. There was not a significant difference in sweet taste of 1B and control samples. 2SB had the lowest aftertaste score. Rest of the samples had similar aftertaste scores.

Overall acceptibility scores showed that the cookie with buttermilk was the most liked one, even more than the control cookies (Figure 3.12). This indicated that buttermilk can be a good ingredient for cookie formulas. Also in the absence of sugar, lactose in buttermilk enhanced the cookie color. 2SB cookies had higher overall acceptability score than 1SB samples. the greatest cohesiveness value was obtained in 2SB followed by control cookies.

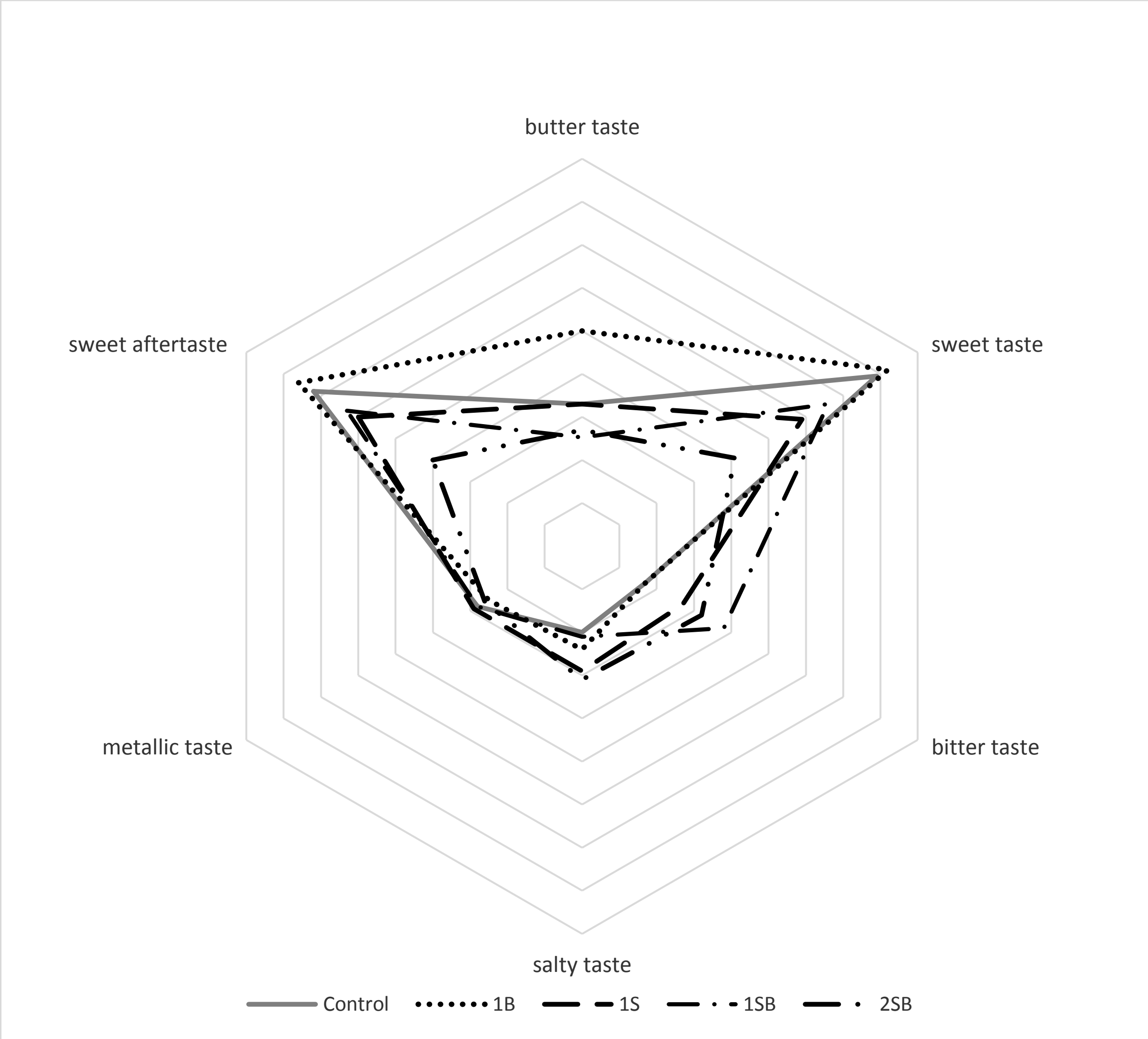


Figure 3.10. Flavor evaluation of cookie samples.

Surface color of 1SB was significantly darker than rest of the samples (Figure 3.11). Control sample was the lightest. Roughness of 2SB was the highest and 1B was the lowest. Inner color of 1SB was the highest and control was the lowest. 1SB and 2SB was similar. Particle size of 1S, 1SB and 2SB was similar and higher than those of 1B and control. Particle size of 1B and control was not different. Crumble in the mouth score of 2SB was the highest and control had the lowest scores. 1SB had the highest hardness score and 1SB, 1S and 2SB was similar. Control had the lowest hardness score. Crispiness of 1S was the greatest and crispiness of 1SB, 1S and 2SB was not different. Control had the lowest hardness score. The score of cohesiveness in mouth was greatest in control and lowest in 1SB. There was no difference in scores of metalic, salty and bitter taste, cohessivines and crumble in the mouth among samples.

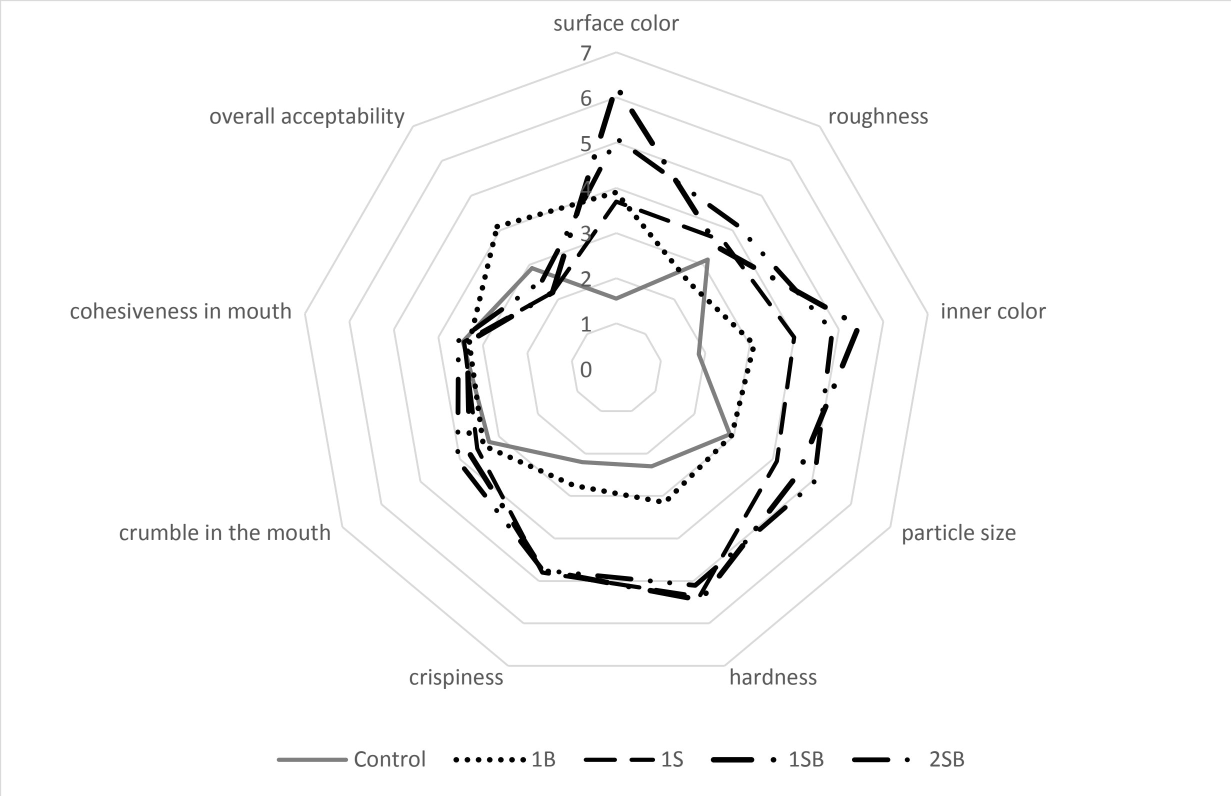


Figure 3.11. Texture evaluation of cookie samples.

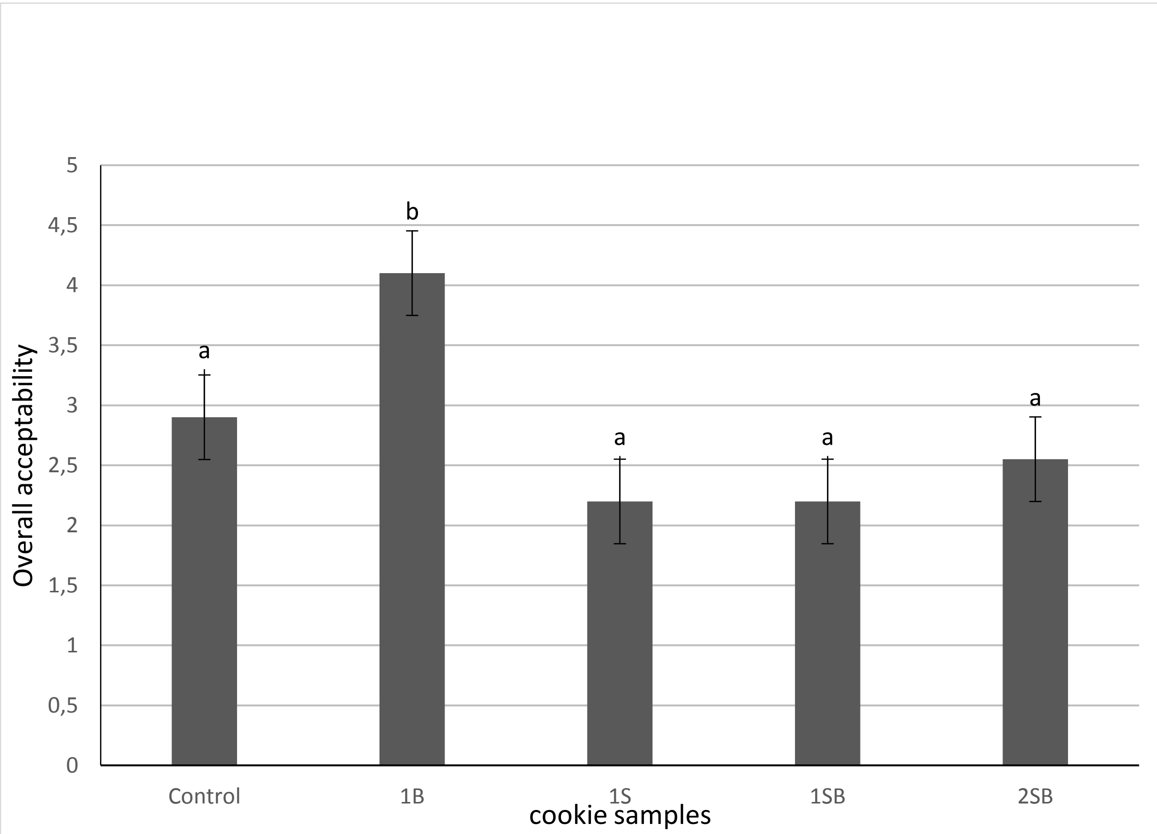


Figure 3.12. Overall acceptability of cookie samples.

3.8. HMF Analysis

2S cookies had the least HMF content (Figure 3.13). Higher HMF amount in 1SB sample can be releated to the baking time, sucrose and lactose. Even the highest HMF content was 20 mg/kg which is in the lower range reported for commercial cookies (0.5- 74.6 mg/kg) (Ameur et al., 2007). There was no significant change in HMF content during storage. 1SB and 2SB had the highest a* and b* values. HMF content was also highest in those samples.

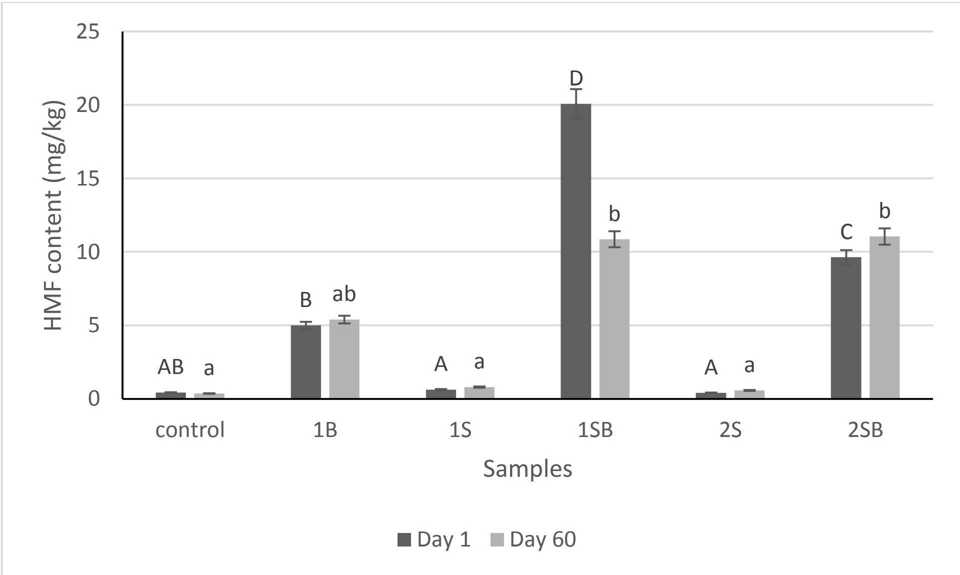


Figure 3.13. HMF content of cookie samples. A-D: Means shown with different letters in the day 1 are significantly different (P<0.05) a-b: Means shown with different letters in day 60 are significantly different (P<0.05)

4. CONCLUSIONS and RECOMMENDATIONS

Spread ratio of sugar-reduced cookies was significantly lower than control and 1B samples. 1B had the highest spread ratio. Moisture content of samples, in the day of baking, was similar. After storage, all of the samples lost significant amount of moisture. On the day of baking, greatest water activity was obtained in 2S samples. After the storage, all the samples with stevia had similar water activity. Water activity values of control and 1B samples were significantly higher than stevia containing cookies. All biscuits exhibited lower L* values than the control biscuit. b* values of all the samples were close to each other. The lowest a* value was found in control cookies. Also when buttermilk powder was introduced to the formula, an increase in a* value was observed in all samples. The total color change of 1SB and 2SB were significantly different than control samples. The hardness values of the samples on the day of baking were close to each other. However, there was an increase and decrease obtained when stevia extract and buttermilk powder was introduced to the formula, respectively. Hardness of 1S and 2S samples was significantly different than control sample. There was not a significant difference between hardness of 1S and 2S samples. There was an increase in hardness of all of the samples after 60 days of storage. Butter taste of 1B sample was significantly higher. 1SB and 2SB had the lowest butter taste score. However, increasing stevia amount did not effect the butter taste. Sweet taste of 1B was the greatest and 2SB was the lowest. Over all acceptibility scores showed that the cookie with buttermilk was the most liked one, even more than the control cookies. Surface color of 1SB was significantly darker than rest of the samples.

The storage study showed that PET containers are not proper for cookie storage by itself. More effective sealing process, an inner package or a different material with more effective vapor-proof property can be more suitable for cookie packaging.

The results showed that buttermilk powder contributed positively to the flavor, texture, color, and moisture of the cookies which makes it an appropriate ingredient for cookies. Buttermilk powder functioned similar to sugar as moisture absorber and it extended the shelf life of cookies. However, it it should be taken into account that buttermilk powder can undergo browning during cooking. Therefore, it can be used in products heat-treated to a limited extent.

4.1. Practical Application of This Study

In this study, sugar reduction was performed with stevia extract in soft cookies. Cookie is one of the high sugar containing foods among bakery products. The study showed that stevia extract can be a good alternative to sugar as a natural sweetener. If sugar reduction with stevia is possible for cookies it could also be suitable for other baked snack foods such as cakes, muffins etc., because these products contain lower amount of sugar compared to that in cookies. The results also showed that buttermilk powder can be a good ingredient in low sugar products.

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&url=http%3A%2F%2Fwww.britishsugar.co.uk%2Ffiles%2Fproductpdfs%2Fscreened_sugars_lo.aspx&usg=AFQjCNGDWePlA8CSIyMFri7ZXZyRuY0gg&sig2=ZpGnfl_QwWBqZmTvm_W8oA&bvm=bv.112064104,d.bGQ&cad=rja,

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APPENDICES

APPENDIX A: Sensory evaluation forms

APPENDIX B: Results of statistical analysis

APPENDIX C: Hplc chromatograms from HMF analysis

APPENDIX D: Graphs obtained in texture analysis

APPENDIX E: Detailed statistical analysis

APPENDIX A: Sensory evaluation forms

TANIMLAYICI DUYUSAL ANALİZ

İsim : Tarih:___/____/______

Sunulan kurabiyeleri tanımları inceledikten sonra tadınız ve formu doldurunuz. Bir kutucuk birden fazla örnek kodu içerebilir. Tadımlar arasında su-kraker-su ile sırasıyla ağzınızı temizleyiniz. Örnekleri size verilen sıraya göre değerlendiriniz.

TAT								
Özellik	**0 Yok**	**1 Çok az**	**2 Az**	**3 Biraz**	**4 Orta**	**5 Biraz fazla**	**6 Fazla**	**7 Çok fazla**
Tereyağı tadı								
Şeker tadı								
Acı (bitter) tat								
Tuzlu tat								
Metalik-genzi yakıcı tat								
Şekerli tadın ağızda kalma süresi								
YAPI								
Özellik	**0 Yok**	**1 Çok az**	**2 Az**	**3 Biraz**	**4 Orta**	**5 Biraz Fazla**	**6 fazla**	**7 Çok fazla**
Kabuk rengi								
Pürüzlülük								
İç renk								
Partikül boyutu								
Sertlik								
Gevreklik								
Ağızda dağılma								
Ağza yapışma								
GENEL BEĞENİ								

Özellik	Tanımı
Kabuk rengi	Kurabiyenin üst yüzeyinin rengi 0: Krem rengi 7: Kahverengi
Pürüzlülük	Ürün yüzeyinde görülen düzensiz boşluk veya tümseklerin yoğunluğu 0: Pürüzsüz 7: Çok pürüzlü
İç renk	Yan kesitten bakıldığında kurabiyenin içinin rengi 0: Krem 7: Kahverengi
Partikül boyutu	Yan kesitten kurabiyenin içine bakıldığında görülen parçacık büyüklüğü 0: Un 7: Toz şeker
Sertlik	İlk ısırışta kurabiyeyi parçalamak için gerek güç 0: Çok yumuşak (kazandibi) 7: Çok sert (galeta)
Gevreklik	İlk ısırışta kurabiyenin kırılabilirliği 0: Taze ekmek içi 7: Galeta
Ağızda dağılma	Kurabiye çiğnenirken ağızda dağılabilirlik, dağılan tanelerin büyüklüğü 0: Taze ekmek içi 7: Un kurabiyesi
Ağzı kaplama	Kurabiye çiğnenirken ağız yüzeyine yapışma düzeyi 0: Kek 7: Un kurabiyesi
Genel beğeni	Numuneyi bütün olarak değerlendiriniz. 0: Hiç beğenmedim 7: Çok beğendim

APPENDIX B: Results of analysis

Table B.1. Results of weight loss, moisture content and water activity analysis

BUTTERMILK POWDER (%)	STEVIA EXTRACT (mL)	WEIGHT LOSS (%)		MOISTURE (%)		WATER ACTIVITY	
		DAY1	DAY60	DAY1	DAY60	DAY1	DAY60
0	0	$6,45\pm0,2^{aA}$	$1,13\pm2,7^{abB}$	$8,28\pm0,4^{aA}$	$5,49\pm0,2^{cB}$	$0,55\pm0,0^{abA}$	$0,42\pm0,0^{cB}$
5	0	$6,65\pm0,15^{abA}$	$0,35\pm0,2^{aB}$	$7,52\pm0,03^{aA}$	$4,93\pm0,2^{abB}$	$0,53\pm0,01^{aA}$	$0,38\pm0,01^{bcB}$
0	7.5	$9,05\pm0^{cA}$	$1,85\pm0,3^{bB}$	$7,74\pm0,1^{aA}$	$4,58\pm0,01^{aB}$	$0,59\pm0^{bcA}$	$0,31\pm0,3^{aB}$
5	7.5	$8,37\pm0,2^{bA}$	$1,03\pm0,4^{abB}$	$7,87\pm0,2^{aA}$	$4,88\pm0,05^{abB}$	$0,57\pm0,01^{abcA}$	$0,35\pm0,03^{abB}$
0	15	$10,56\pm0,06^{dA}$	$0,78\pm0,4^{aB}$	$8,60\pm0,57^{aA}$	$5,56\pm0,1^{cB}$	$0,64\pm0,0^{dA}$	$0,32\pm0,0^{aB}$
5	15	$9,88\pm0,4^{eA}$	$0,95\pm0,4^{abB}$	$8,47\pm1^{aA}$	$5,14\pm0,4^{bcB}$	$0,61\pm0,02^{dcA}$	$0,31\pm0,02^{aB}$

a-e: Means shown with different letters in the same column are significantly different ($P<0.05$)
A-E: Means shown with different letters in the same row for each property are significantly different ($P<0.05$)

Table B.2. Results of texture and spread ratio analysis

BUTTERMILK POWDER (%)	STEVIA (mL)	HARDNESS (N)		WORK (N mm)		SPREAD RATIO
		Day1	Day60	Day1	Day60	Day1
0	0	15,20±2,4^{aA}	76,94±5,1^{aB}	53,37±8,5^{cA}	83,64±43,1^{aA}	4,14±0,3^{c}
5	0	20,81±2,4^{abA}	84,93±10,1^{abB}	66,62±4,7^{dA}	126,21±90,4^{aA}	4,47±0,1^{d}
0	7.5	29,94±1,4^{bcA}	99,97±5,9^{abB}	110,87±3,3^{abA}	134,59±134,5^{aA}	3,37±0^{ab}
5	7.5	14,73±1,4^{aA}	85,08±11,7^{abB}	64,06±3,6^{abA}	172,88±88,09^{aA}	3,26±0,01^{ab}
0	15	32,23±7,8^{cA}	106,21±14,4^{bB}	154,72±10^{bA}	136,85±100^{aA}	3,55±0,07^{b}
5	15	17,29±5,3^{aA}	75,99±17,8^{aB}	103,36±24,9^{aA}	168,20±85,4^{aA}	3,10±0,08^{a}

a-e: Means shown with different letters in the same column are significantly different (P<0.05)
A-E: Means shown with different letters in the same row for each property are significantly different (P<0.05)

Table B.3. Results of color analysis

BUTTERMILK POWDER(%)	STEVIA (mL)	COLOR						TOTAL COLOR CHANGE (ΔE*)	
		L*		a*		b*		Day1	Day60
		Day1	Day60	Day1	Day60	Day1	Day60		
0	0	$73,02\pm1,6^{cA}$	$79,64\pm1,7^{cB}$	$1,98\pm1,3^{aA}$	$0,19\pm1,3^{aA}$	$30,2\pm1^{aA}$	$35,01\pm2,1^{aB}$	-	-
5	0	$63,45\pm1,6^{bA}$	$71,27\pm4^{bA}$	$6,91\pm1,5^{bA}$	$5,35\pm1,9^{bA}$	$32,7\pm4,2^{abA}$	$40,20\pm1,1^{bcA}$	$11,05\pm0,3^{aA}$	$10,9\pm4,5^{aA}$
0	7.5	$64,26\pm0,4^{bA}$	$70,09\pm0,4^{bB}$	$5,39\pm0,3^{bA}$	$4,68\pm0,4^{bA}$	$33,40\pm0,2^{abA}$	$39,60\pm0,4^{bcB}$	$9,92\pm1,06^{aA}$	$8,53\pm0,6^{aA}$
5	7.5	$56,09\pm1,1^{aA}$	$62,22\pm2,02^{aB}$	$10,34\pm0,4^{cA}$	$10,18\pm0,89^{cA}$	$35,87\pm0,6^{bA}$	$42,40\pm0,44^{cB}$	$19,71\pm0,79^{bA}$	$8,96\pm1,9^{bA}$
0	15	$63,52\pm0,5^{bA}$	$68,55\pm0,7^{bB}$	$5,09\pm0,5^{bA}$	$4,90\pm0,4^{bA}$	$33,11\pm0,4^{abA}$	$39,00\pm0,7^{bB}$	$10,40\pm2,08^{aA}$	$7,75\pm0,95^{aA}$
5	15	$56,80\pm1^{aA}$	$63,33\pm1,9^{aB}$	$9,10\pm0,7^{cA}$	$8,89\pm1,3^{cA}$	$35,16\pm0,5^{bA}$	$41,09\pm1,4^{bcB}$	$19,71\pm1,65^{bA}$	$8,82\pm1,8^{bA}$

a-e: Means shown with different letters in the same column are significantly different ($P<0.05$)
A-E: Means shown with different letters in the same row for each property are significantly different ($P<0.05$)

Table B.4. Results of HMF analysis

BUTTERMILK POWDER (%)	STEVIA (mL)	HMF (mg/kg) Day1	HMF (mg/kg) Day60
0	0	0,43±0,04^{abA}	0,36±0,04^{aA}
5	0	5,00±2,50^{bA}	5,39±2,81^{abA}
0	7.5	0,63±0,08^{aA}	0,79±0,07^{aA}
5	7.5	20,08±0,48^{dA}	10,75±3,06^{bA}
0	15	0,41±0,04^{aA}	0,56±0,00^{aA}
5	15	9,64±0,45^{cA}	10,94±4,08^{bA}

a-e: Means shown with different letters in the same column are significantly different ($P<0.05$)

A-E: Means shown with different letters in the same row for each property are significantly different ($P<0.05$)

APPENDIX C: Hplc chromatograms from HMF analysis

Appendix C.1: HMF peak of samples at day1

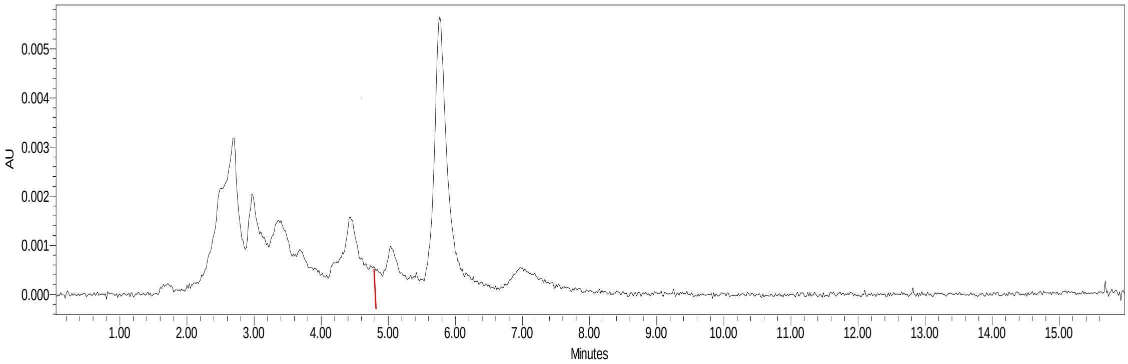

Figure C.1.1. Control sample

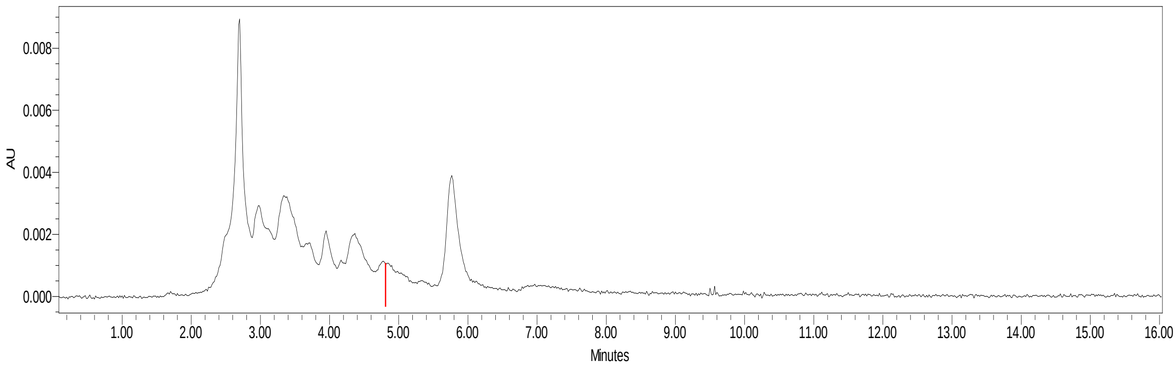

Figure C.1.2. 1B sample

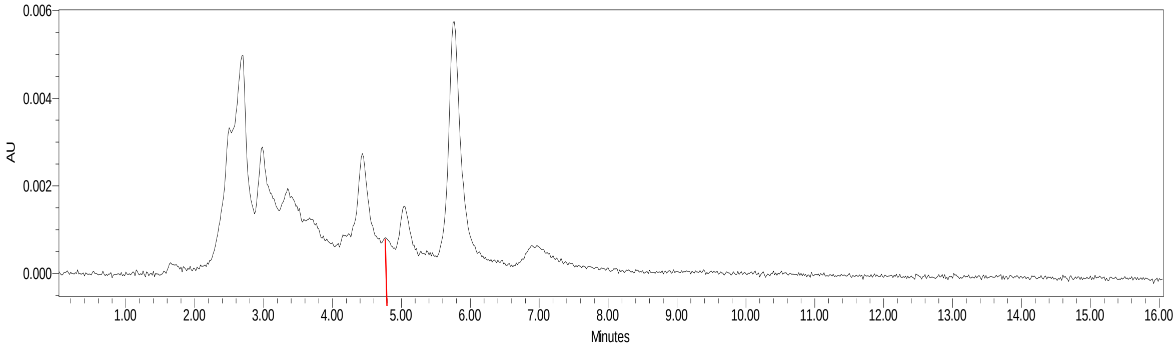

Figure C.1.3. 1S sample

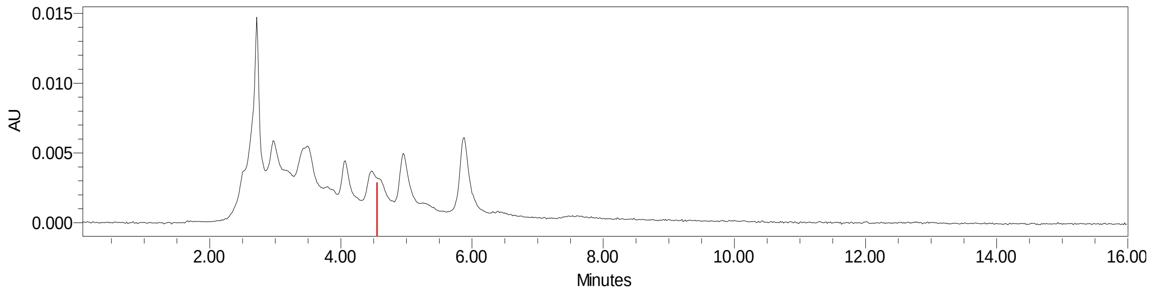

Figure C.1.4. 1SB sample

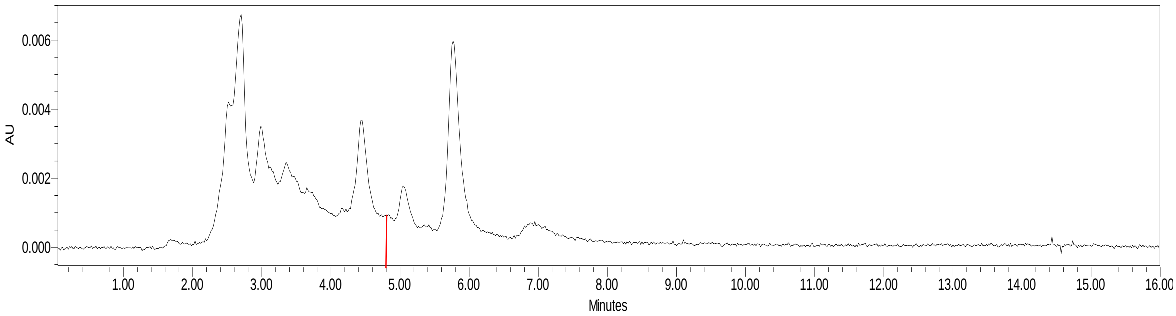

Figure C.1.5. 2S sample

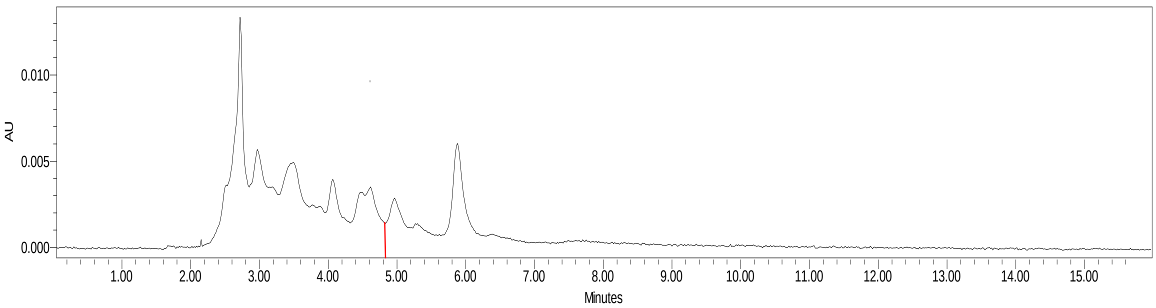

Figure C.1.6. 2SB sample

Appendix C.2 HMF peak of samples at day 60

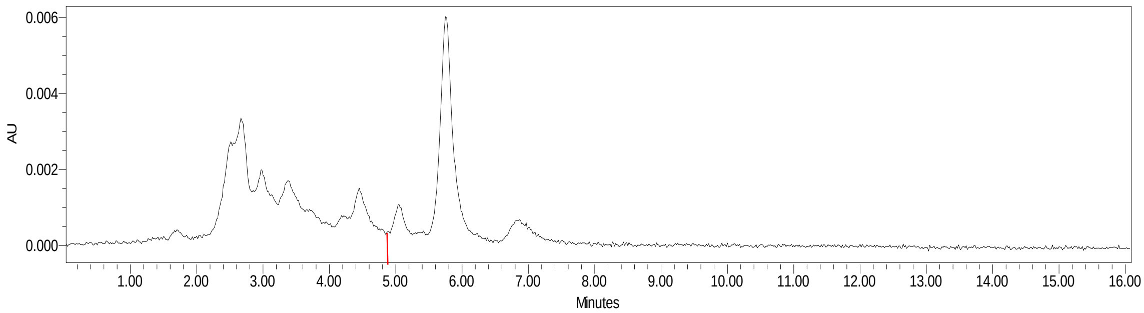

Figure C.2.1. Control sample

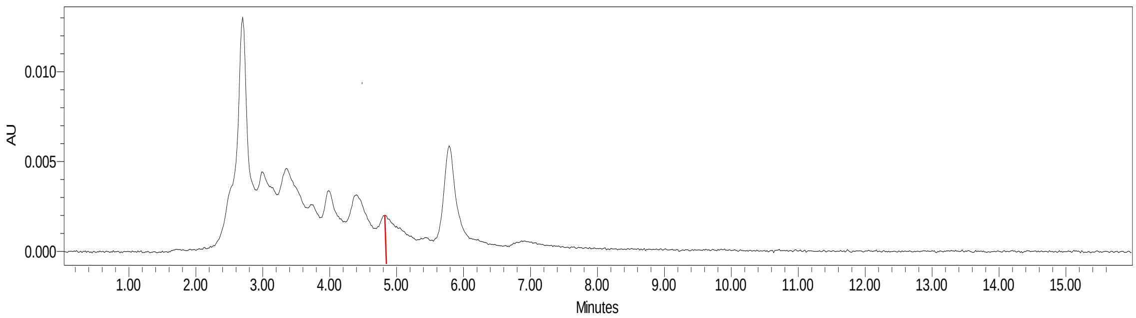

Figure C.2.2.1B sample

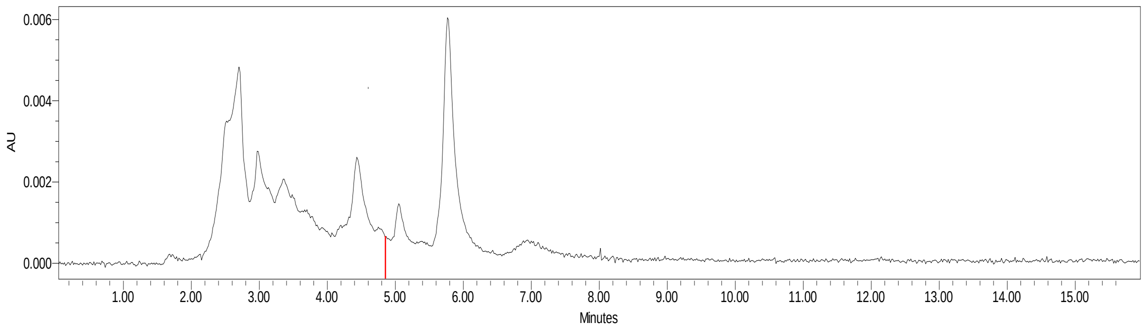

Figure C.2.3. 1S sample

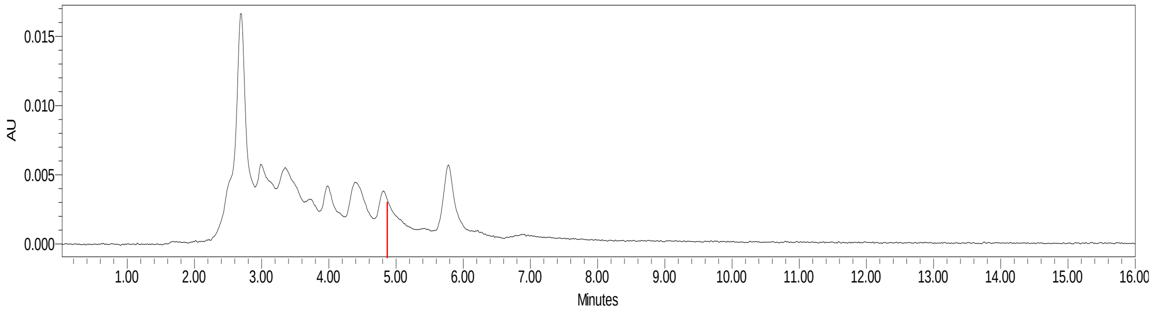

Figure C.2.4. 1SB sample

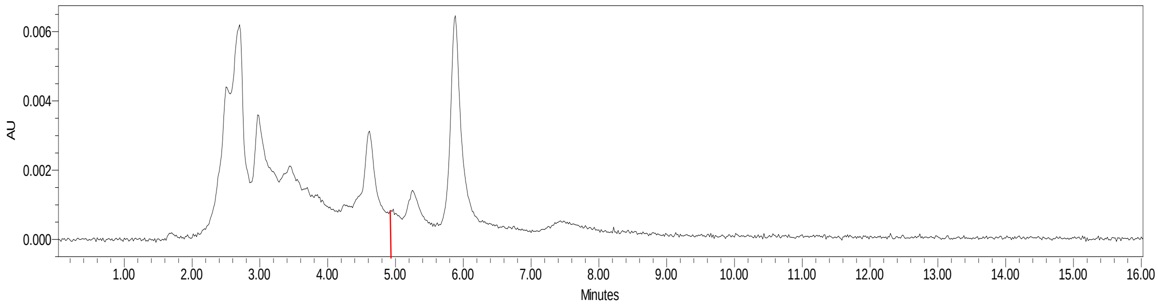

Figure C.2.5. 2S sample

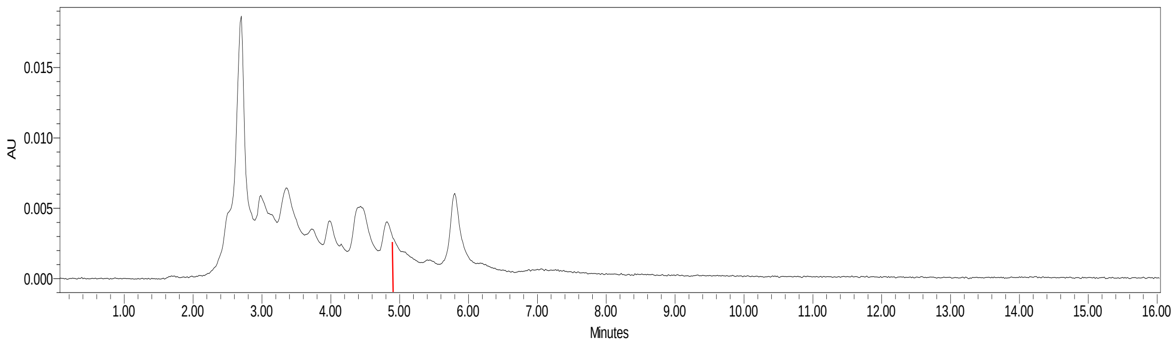

Figure C.2.6. 2SB sample

APPENDIX D: Graphs obtained in texture analysis

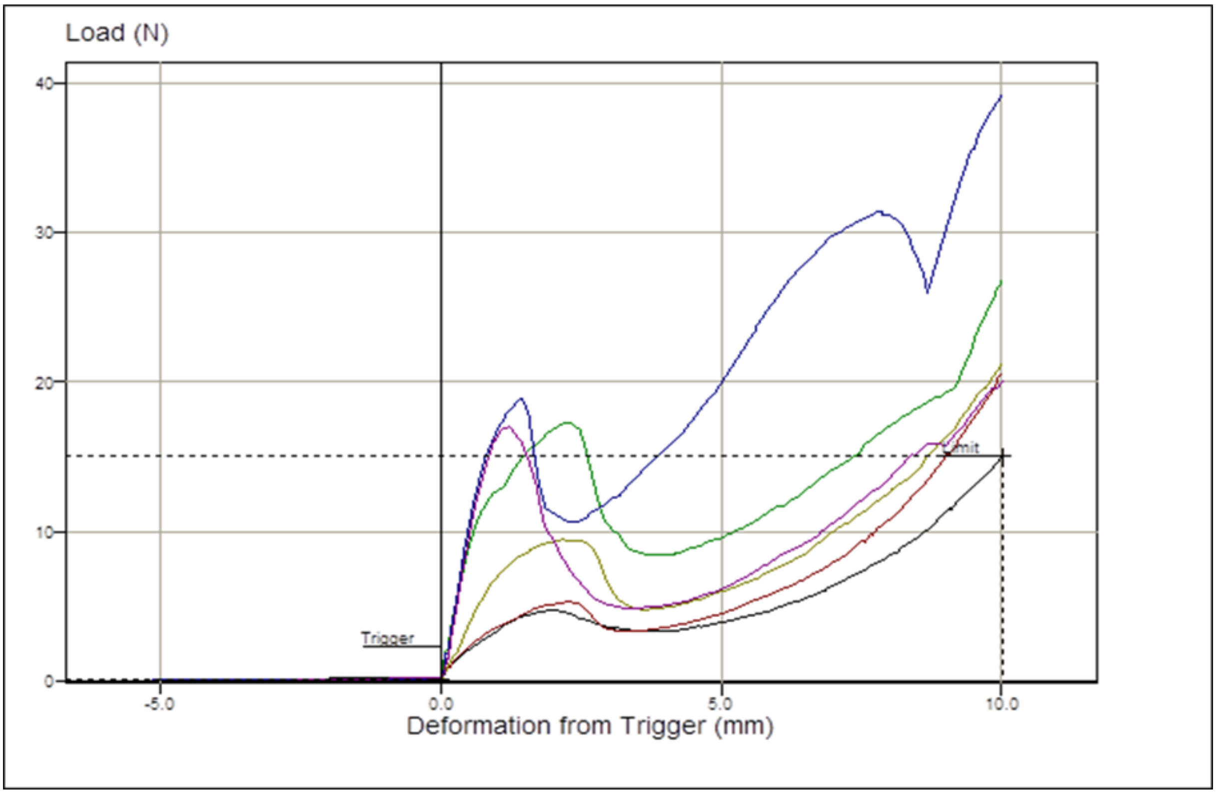

Figure D.1.Texture analysis graph of day1

control

1B

1S

1SB

2S

2SB

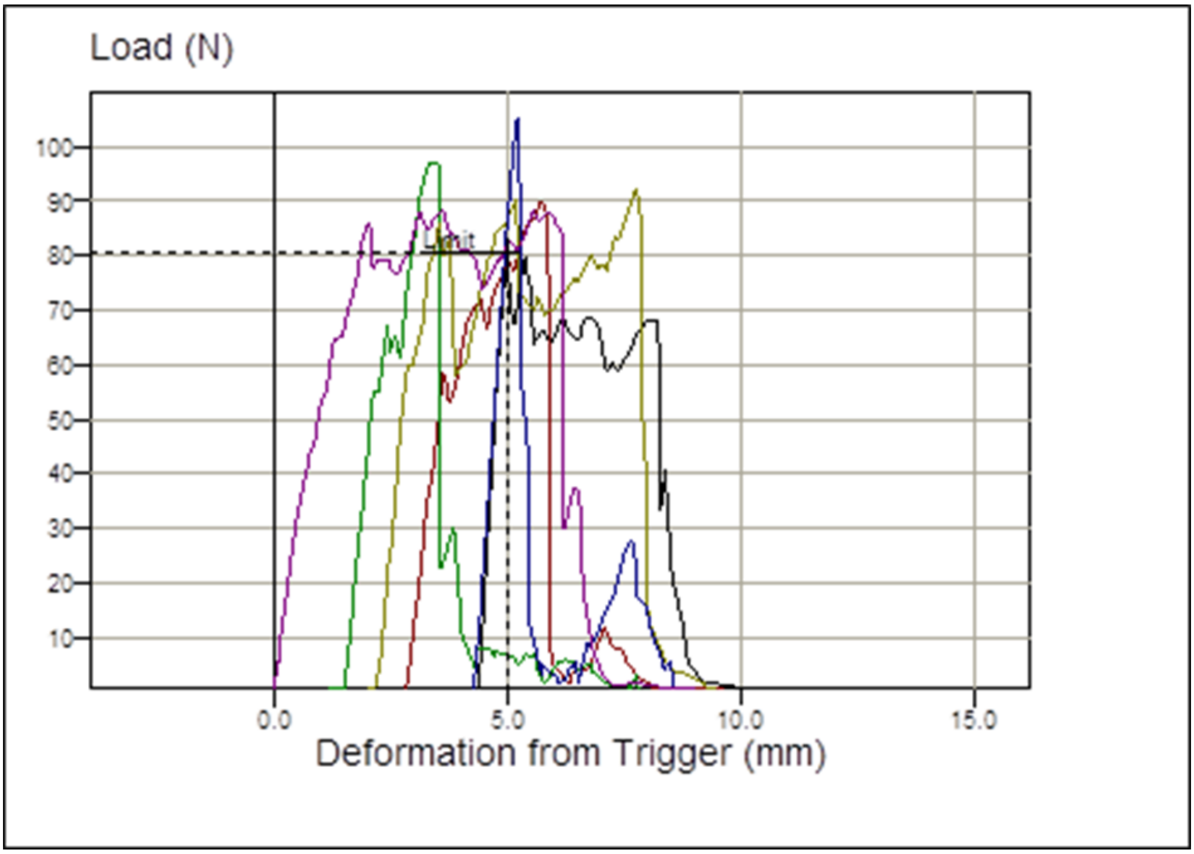

Figure D.2: Texture analysis graph of day60

control

1B

1S

1SB

2S

2SB

APPENDIX E: Detailed results of statistical analysis

Table E.1. p-values of factors

Factors	**Properties**									
	Water Activity	Weight Loss	L	a	b	Spread	Work	Moisture content	Hardness	HMF
Stevia	.000	.000	.014	NS	NS	.000	.001	NS	NS	NS
Buttermilk	NS	NS	.001	.000	NS	NS	NS	NS	.033	.000
Time	.000	NS	.024	NS	.000	NS	NS	.000	.000	NS
Stevia*Buttermilk	NS	.045	NS	NS	NS	.011	.004	NS	.006	NS
Stevia* Time	.000	.000	NS	NS	NS	NS	NS	NS	NS	NS
Buttermilk*Time	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant

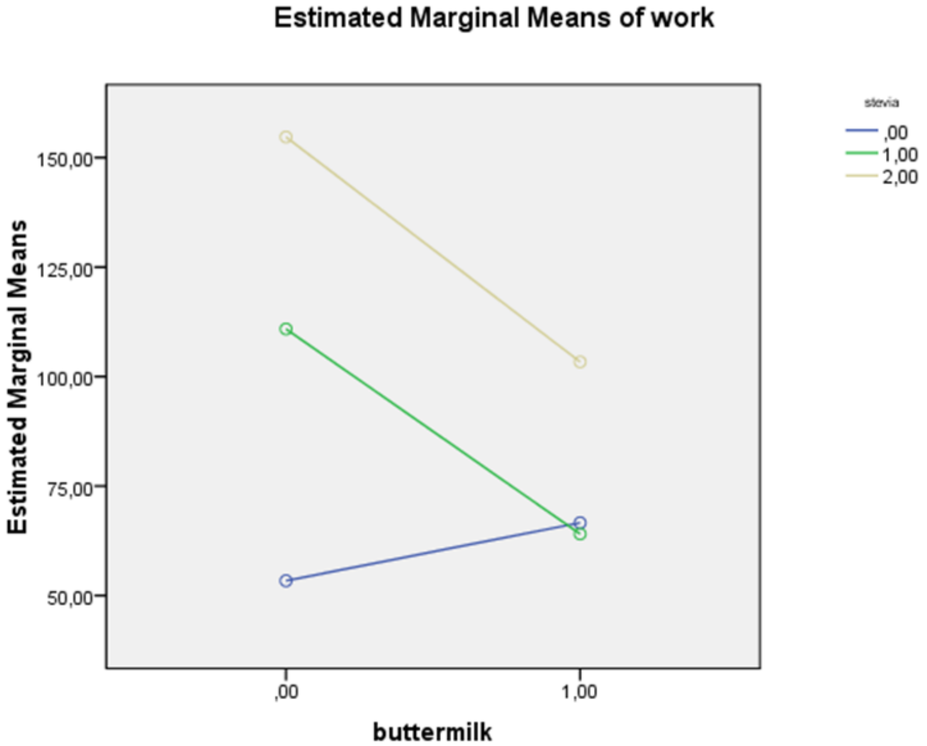

Figure E. 1. Interaction of buttermilk and stevia on work value

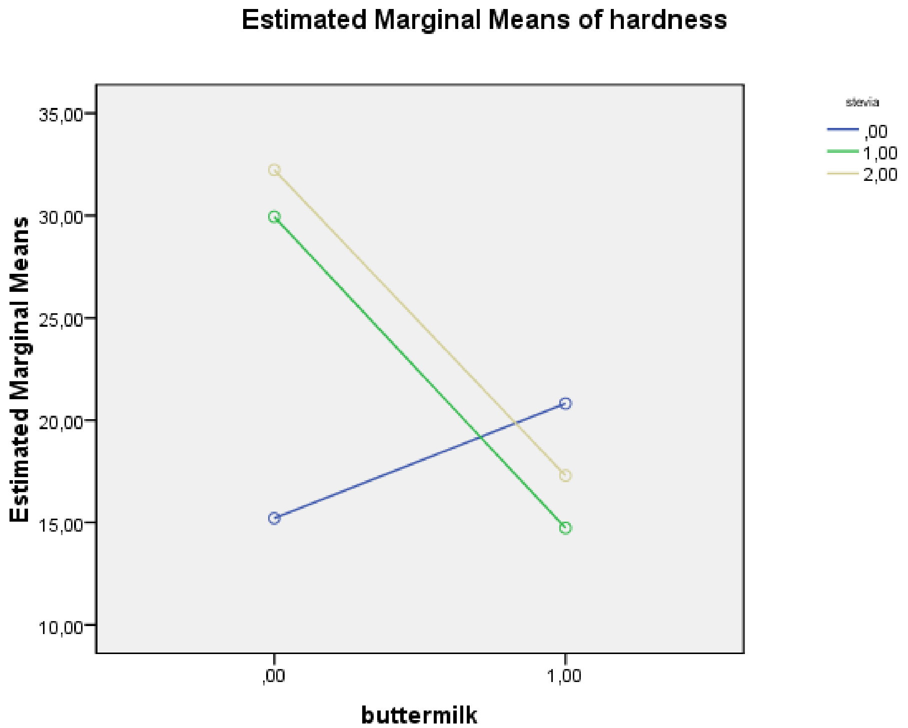

Figure E.2. Interaction of buttermilk and stevia on hardness

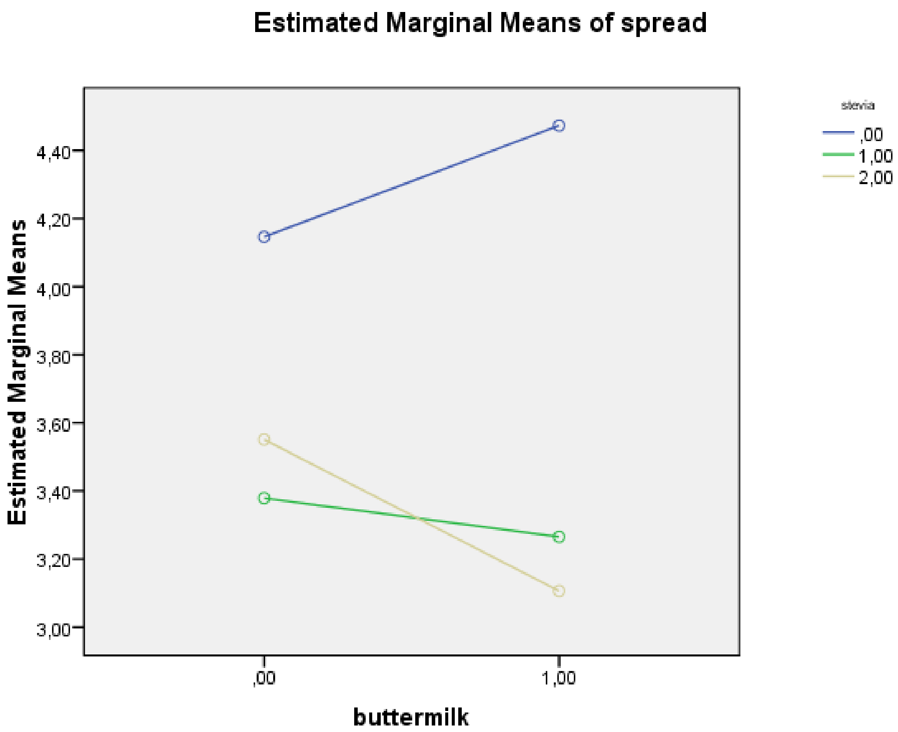

Figure E.3. Interaction of buttermilk and stevia on spread

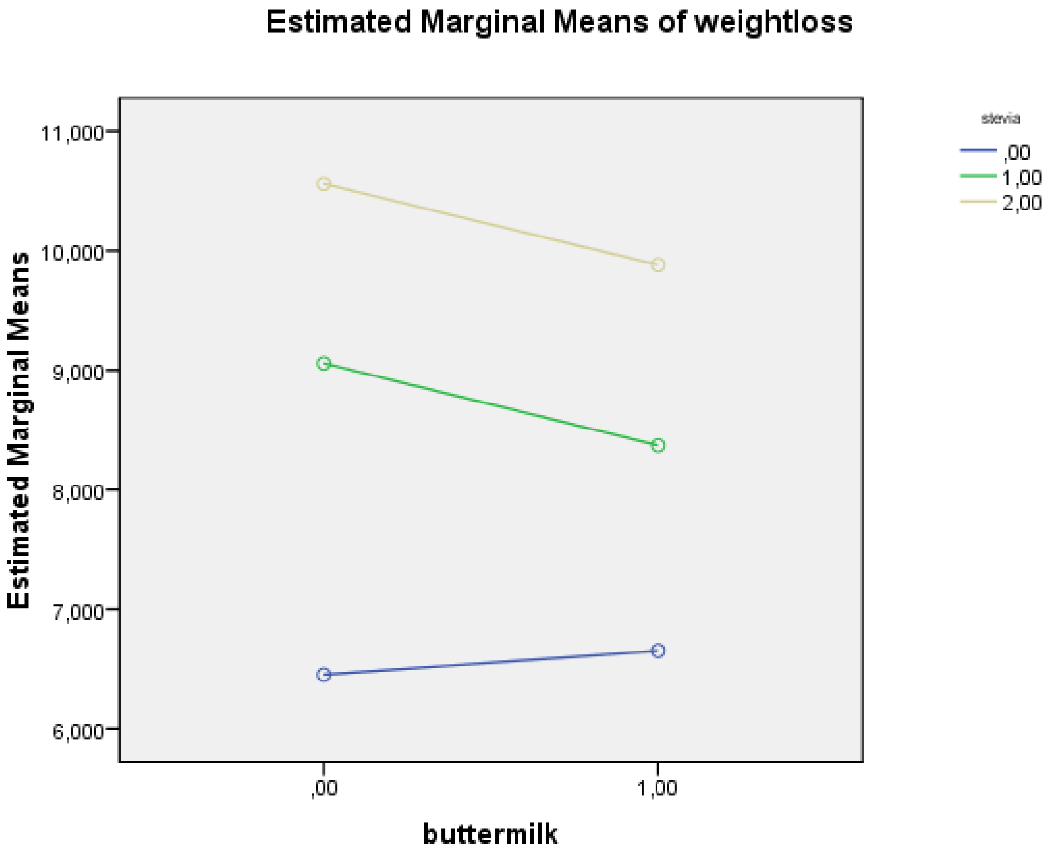

Figure E.4. Interaction of buttermilk and stevia on weightloss

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